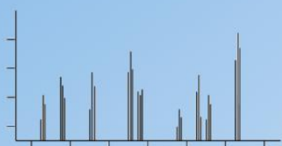

Mass Spectrometry Group
Institute of Organic Chemistry, Polish Academy of Sciences

33rd INFORMAL MEETING ON MASS SPECTROMETRY

10th to 13th May 2015

Szczyrk, Poland

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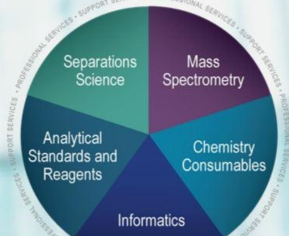

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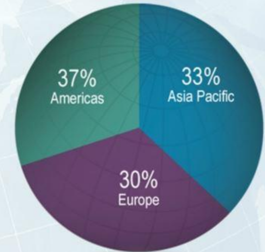

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&

Unleash
The Science
to make it a reality

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33rd Informal Meeting on Mass Spectrometry

10th - 13th May, 2015 Szczyrk, POLAND

organized by:
Institute of Organic Chemistry, Polish Academy of Sciences and Polish Mass Spectrometry Society

Chairman:

Witold Danikiewicz (Institute of Organic Chemistry, Polish Academy of Sciences)

Co-chairmen:

Pietro Traldi (CNR-IENI, Padova, Italy) and Karoly Vekey (Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary)

Organizing Committee:

Witold Danikiewicz (Chairman), Paweł Świder (Secretary), Kacper Błaziak, Magdalena Kania, Marian Olejnik, Barbara Repeć, Przemysław Sendys, Maciej Sojka, Grzegorz Spólnik, Anna Troć, Magdalena Zimnicka

Venue:

Hotel Meta, ul. Skośna 4, 43-370 Szczyrk, Poland

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CONFERENCE PROGRAM

Sunday, May 10th, 2015

	15:00 – 19:00	*Registration*
	19:00 –	*Get-together party*

Monday, May 11th, 2015

	8:00 – 8:45	*Registration*
		Chairman: W. Danikiewicz
	8:45 – 9:00	Opening
MoOr01	9:00 – 9:20	**M. Claeys, M. S. Shalamzari, R. Vermeylen, F. Blockhuys, T. E. Kleindienst, M. Lewandowski, R. Szmigielski, K. Rudziński, G. Spólnik, W. Danikiewicz and W. Maenhaut** Characterization of secondary organic aerosol from green leaf volatiles at the molecular level using mass spectrometric approaches
MoOr02	9:20 – 9:40	**R. Szmigielski, P.Wach-Panfiłow, G. Spólnik, K. Szmigielska, K. J. Rudziński** Electrospray tandem mass spectrometry of aqueous-phase isoprene-derived secondary organic aerosols
MoOr03	9:40 – 10:00	**F. L. Bazsó, P. Janzsó, O. Ozohanics, L. Drahos** Information content of tandem mass spectra
MoOr04	10:00 – 10:20	**A. Maroto, T. Josse, R. Lartia, J. De Winter, P. Gerbaux, A. Memboeuf** Energy-resolved tandem mass spectrometry for the structural and quantitative analysis of isobaric/isomeric mixtures
	10:20 – 11:00	*Coffee break*
		Chairman: P. Derrick
MoOr05	11:00 – 11:20	**M. Waliczek, M. Kijewska, M. Rudowska, A. Kluczyk, P. Stefanowicz, Z. Szewczuk** Peptides labeled with pyridinium salts for sensitive sequencing by electrospray tandem mass spectrometry
MoOr06	11:20 – 11:40	**J. Novák, V. Havlíček** De novo identification of nonribosomal peptides from accurate product ion mass spectra

MoOr07	11:40 – 12:00	**L. Mark, Z. Papai, K. Banfai, J. Schmidt** The embryome: molecules in space and time during the early stage development
MoOr08	12:00 – 12:20	**M. Kennedy, B. Czerski** Ion mobility mass spectrometry, from research to routine
	12:20 – 14:00	*Lunch*

Chairman: F. De Angelis

MoOr09	14:00 – 14:20	**P. J. Derrick, N. J. Demarais, D. Greenwood, A. Radionova** Investigation of protein behaviour on microsecond time-scales using dual-channel nano-electrospray emitters
MoOr10	14:20 – 14:40	**G. Grasso** The application of mass spectrometry for the analysis of the cryptic peptides produced by the action of IDE on some of its substrates
MoOr11	14:40 – 15:00	**M. Modzel, H. Płóciennik, P. Stefanowicz** A synthesis of new, bi-labelled peptides for quantitative proteomics
	15:00 – 16:30	*Coffee break and Poster session 1*

Chairman: D. Kuck

MoOr12	16:30 – 16:50	**J.-C. Tabet, E. Darii, S. Alves, A. Perret** Basicity and acidity influence on the stabilization of the hydrogen bond and salt bridge competitive interactions in anionic arginine/hexose-phosphate complexes
MoOr13	16:50 – 17:10	**F. Tisato, V. Peruzzo, M. Porchia, C. Santini, M. Pellei, P. Traldi** ESI-MS^n study of the interaction products of a cytotoxic phosphino copper(I) complex with methionine-rich peptides
MoOr14	17:10 – 17:30	**W. Szkutnik** Fundamentals and Advances of Orbitrap Mass Spectrometry – applications and current trends in analysis
	19:30 –	*Grill party*

Tuesday, May 12th, 2015

Chairman: K. Vekey

TuOr01	9:00 – 9:20	**C. Iacobucci, J.-F. Gal, S. Reale, F. De Angelis** Studying organic reactions mechanism by ESI-MS: the case of the Ugi and Ugi-Smiles reactions
TuOr02	9:20 – 9:40	**K. J. Jobst, J. K. Terlouw, T. Luider, N. A. van Huizen, P. C. Burgers** Interaction of metal cations with alkylnitriles, alkylamines and alkylalcohols in the gas-phase: solvation of metal ions by the hydrocarbon chain
TuOr03	9:40 – 10:00	**D. Kuck, D. Yang, B. Xia, Y. Jiang, W. Mei** Fragmentation of protonated 2-(2-phenylethyl)chromones: the diagnostic role of ion/neutral complexes
TuOr04	10:00 – 10:20	**B. Repeć, K. Błaziak, W. Danikiewicz** Gas-phase reactions of methyl thiocyanate and dimethyl disulfide with carbanions
	10:20 – 11:00	*Coffee break*

Chairman: P. Traldi

TuOr05	11:00 – 11:20	**H. Hevér, E. Tóth, O. Ozohanics, A. Telekes, K. Vékey, L. Drahos** Correcting long-term drifts in HPLC-MS runs
TuOr06	11:20 – 11:40	**T. Pluháček, K. Lemr, J. Novák, O. Benada, L. Krásný, J. Pól, M. Volný, M. Strohalm, V. Havlíček** Imaging mass spectrometry
TuOr07	11:40 – 12:00	**K. Pawlak, M. Kupiec,W. Jakubczak, M. Matczuk, M. Jarosz** Mass spectrometry “on the track” of metallodrugs’ metabolism
TuOr08	12:00 – 12:20	**P. Tarnowski** Answers for Science. Knowledge for Life.™
	12:20 – 14:00	*Lunch*

Chairman: V. Havlíček

TuOr09	14:00 – 14:20	**P. Gerbaux, G. Carroy, V. Lemaur, J. De Winter, J. Cornil** Supramolecular mass spectrometry: association of MS methods to computational chemistry to access, at a molecular level, systems relevant to host-guest chemistry
TuOr10	14:20 – 14:40	**I. D. Kellner, T. Drewello** Influence of single skimmer vs. dual funnel transfer on the appearance of ESI-generated LiCl cluster / ß-cyclodextrin inclusion complexes
TuOr11	14:40 – 15:00	**M. Witt** Isotopic fine structure – a perfect tool for unambiguous molecular formula elucidation
	15:00 – 16:30	*Coffee break and Poster session 2*

Chairman: Z. Szewczuk

TuOr12	16:30 – 16:50	**C. Iacobucci, S. Reale, J-F Gal, F. De Angelis** Copper(I)-catalyzed azide–alkyne cycloaddition: an ESI-MS(/MS) mechanistic investigation
TuOr13	16:50 – 17:10	**R.W. Kirschbaum, D. Prenzel, S. Frankenberger, R.R. Tykwinski, T. Drewello** Gas-phase experiments with polyynes: laser activation and Ag^{+}-induced reactivity
TuOr14	17:10 – 17:30	**E. Fornal** Application of LC/QTOF in analysis of drugs, drugs candidates and new psychoactive substances
	19:30 –	*Conference dinner*

Wednesday, May 13th, 2015

Chairman: P. Stefanowicz

WeOr01	9:00 – 9:20	**J. Far, C. Kune, D. Cédric, E. Hanozin, D. Morsa, E. De Pauw** Probing the liquid to gas phase structural modifications of ions
WeOr02	9:20 – 9:40	**I. Czerwinska, J. Far, D. Morsa, N. Smargiasso, E. De Pauw** Ruthenium-arene complexes studied by ion mobility mass spectrometry combined with CID technique
WeOr03	9:40 – 10:00	**J.F. Hitzenberger, C. Damman, N. Lang, D. Lungerich, G. Bottari, N. Jux, T. Torres, T. Drewello** Making the invisible visible: formate attachment to divalent metalloporphyrins
WeOr04	10:00 – 10:20	**M. Zimnicka, M. Koliński, J. L. Sessler** Gas-phase association modes of monopyrene tweezers through experimental and theoretical approaches
	10:20 – 11:00	*Coffee break*

Chairman: M. Jarosz

WeOr05	11:00 – 11:20	**M. Olejnik, L. Radko, B. Korycińska, P. Jedziniak** Identification of metabolites of polyether antibiotic salinomycin using Q-TOF and hybrid QQQ-linear ion trap
WeOr06	11:20 – 11:40	**P. Pomastowski, B. Buszewski** Immobilization of silver onto lactoferrin in light of new antimicrobial application
WeOr07	11:40 – 12:00	**P. Stalica** Multi-dimentional chromatographic systems coupled with mass spectrometry
	12:00 – 12:20	*Concluding remarks and invitation to the 34th IMMS*
	12:20 – 14:00	*Lunch*
	15:00 – 18:00	Excursion to the top of the Skrzyczne mountain (up by a chair lift, down either by a chair lift or on foot).

POSTERS

Monday Posters – May 11, 2015		
MoPo01	**Zrinyi, Z., Petrovics, D., Rivnyak, A., Tamas, A., Mark, L., Kiss, T., Reglodi, D., Pirger, Z., Maasz, G.**	Evolutionarily conserved neuroprotective function of pituitary adenylate cyclase-activating polypeptide (PACAP) in dopamine-based neurodegeneration
MoPo02	**B. Jakob, E. Kristóf, K. Csomós, L. Fésüs and É. Csősz**	Analysis of the protein crosslink-profile changes during neutrophil extracellular trap formation
MoPo03	**Z. Timár, É. Molnár, P. Pádár, E. Beéry, A. Csorba**	Generic LC-MS/MS quantification for biowaiver pharmacokinetic transport assays
MoPo04	**G. Bartkowiak, K. Tadyszak, S. Jurga, G. Schroeder**	Mass spectrometric detection of nitric oxide release from S-nitrosoglutathione and S-nitroso-N-acetyl-DL-penicillamine
MoPo05	**A.Godziek, A. Maciejowska, T. Kowalska, E. Talik, M. Sajewicz**	HPLC-MS investigation of nano- and microstructures formed from monomeric amino acids phenylglycine and cysteine as a result of oscillatory reaction
MoPo06	**A. Maciejowska, A. Godziek, M. Sajewicz, T. Kowalska**	Spontaneous oscillatory reaction of protein amino acids in an abiotic system – the LC-MS results
MoPo07	**M. Szala, K. Czyż, R. Jędrszczyk, K. Kuna, M. Zawiązalec, T. Paździorek and J. E. Nycz**	MS spectra for dyes hydroxyazoquinoline derivatives
MoPo08	**A. Nowok, M. Piernik, J.E. Nycz**	Synthesis of selected 1,10-phenanthrolines by Skraup reaction; GC-MS investigation
MoPo09	**K. Czyż, M. Szala, R. Jędrszczyk, K. Kuna, M. Zawiązalec, T. Paździorek and J. E. Nycz**	Synthesis of selected 1,10-phenantrolines; GC/MS investigation
MoPo10	**P.Marczewska, D. Szeremeta, J. Rzepa, M. Sajewicz**	GC-MS in analysis of lavender volatile components
MoPo11	**D. Szeremeta, P. Marczewska, J. Rzepa, M. Sajewicz**	Identification of hexachlorocyclohexane biodegradation products by GC-MS
MoPo12	**W. Ostrowski and R. Frański**	Formation of curcumin molecular ion under electrospray ionisation conditions in the presence of metal cations
MoPo13	**V. Pashynska, S. Stepanian, M. Kosevich, A. Gomory and K.Vekey**	Combined model mass spectrometric and quantum chemical study of artemisinin-type agents and aspirin interactions with membrane phospholipids
MoPo14	**M. Zimnicka**	Probing molecular recognition by mass spectrometry. A case study: examination of properties of noncovalent complexes of selected melanocortin receptor ligands
MoPo15	**S. Golba and J. Gabor**	MALDI-TOF spectra of conducting polymers

MoPo16	**M.Cvijovic, B.Nastasijevic, M.Marceta Kaninski, M.Zivkovic**	GC MS and ICP MS determination of restauration formulation used for fireplace clocks
MoPo17	**M. Maksymiak, T. Bałakier, J. Jurczak, M. Kowalczuk, G. Adamus**	Molecular architecture of novel biodegradable and biocompatible control delivery systems of selected antioxidants established by electrospray ionization multistage mass spectrometry
MoPo18	**P.Sendys and W. Danikiewicz**	Gas-phase reactions of tert-butyl and cumene peroxide anions with alkyl and allyl halides
MoPo19	**K. Macur, P. Czaplewska, I. Behrendt, M. Prądzińska**	Characterization of intact anti-human cystatin C antibodies, Cyst10 and Cyst28, using the µLC-MS/MS TRIPLETOF 5600+ system
MoPo20	**M. Piątkowska, P. Jedziniak and J. Żmudzki**	Simultaneous determination of ten illegal dyes in animal feed by high performance liquid chromatography coupled with tandem mass spectrometry
MoPo21	**M. Antczak and K. Mitrowska**	Impact of dwell, pause, scan event, and, loop times on measurement possibilities in multiresidue methods
MoPo22	**K. Błaziak and W. Danikiewicz**	From proton bonded dimers to absolute proton affinity values (PA) of aromatic carboxylic acids and appropriate phenide anions. Theoretical study based on DFT and multilevel calculations.
MoPo23	**A. Lewandowska , K. Macur, P. Czaplewska , S. Ołdziej**	Optimisation of the human blood serum fractionation method for the SWATH-MS analysis
MoPo24	**M. Roś, M. Pietrowska, P. Widłak**	An optimization of the MALDI-TOF analysis method for serum lipidome mass profiling in cancer research
MoPo25	**M. Majchrzak, M. Rojkiewicz, A. Mazurkiewicz, R. Celiński, Mieczysław Sajewicz**	Novel derivatives of cathinone contained in designer drugs- chromatographic and spectroscopic methods for identification
MoPo26	**T. Neugebauer and T. Drewello**	Formation and dissociation behaviour of salt-like cluster ions (sodium formate) in ESI-ion trap experiments
MoPo27	**V.G. Zobnina, M.V. Kosevich, V.V. Chagovets and O.A. Boryak**	Clustering of nitrogen bases with polyethylene glycol: electrospray mass spectrometry and computer experiment
MoPo28	**Z. Papai, J. Schmidt, P. Avar, K. Banfai, T. Bakk, L. Mark**	Maternal-embryo lipid networking during the early stage embryogenesis
MoPo29	**E. Jambor, J. Schmidt, A. Bona, T. Tenyi, L. Mark**	New salivary biomarkers of schizophrenia
MoPo30	**K. Banfai, T. Jarai, J. Schmidt, L. Mark**	Local expression of proteins in the tumor microenvironment of head neck squamous cell carcinoma

MoPo31	**G. Kalló, T. Székely, J.A. Mótyán, Sz. Benkő, J. Tőzsér and É. Csősz**	Relative quantification of the human Nod-like receptor family card domain containing 5 (NLRC5) protein by targeted mass spectrometry approach
MoPo32	**M.V. Kosevich, V.S. Shelkovsky, O.A. Boryak, V.G. Zobnina, V.S. Leontiev, V.V. Orlov, V.V. Karachevtsev, O.V. Severinovskaya and V.A. Pokrovskiy**	Mass spectrometric search for silver nanoclusters in carbon nanotubes-silver composite
MoPo33	**Peter Avar**	Cell energetics with HPLC-MS
MoPo34	**M. Demeyer, E. Hennebert, G. Caulier, J. De Winter, M. Wisztorski, I. Fournier, I. Eeckhaut, P. Flammang and P. Gerbaux**	Molecular diversity and body distribution of saponins in the sea star *asterias rubens* by mass spectrometry
MoPo35	**T. Josse, J. De Winter, P. Dubois, O. Coulembier, A. Memboeuf and P. Gerbaux**	A tandem mass spectrometry-based method to assess the architectural purity of synthetic polymers: a case of a cyclic polylactide obtained by click chemistry
MoPo36	**A. Troć, M. Zimnicka, M. Koliński, W. Danikiewicz**	Analysis of β-lactams by ion mobility – mass spectrometry and theoretical calculations

Tuesday Posters – May 12, 2015

TuPo01	**M. Łuczak, Ł. Marczak, M. Stobiecki**	Mass spectrometry-based relative quantitation of chronic kidney disease plasma proteins using iTRAQ and label-free strategies
TuPo02	**M. Kalinowska-Herok, M. Pietrowska, M. Gawin, M. Chekan, J. Wierzgon, G. Drazek, J. Polanska, A. Polanski, P. Widlak**	Molecular characterization of oral squamous cell cancer and adjacent tissues by MALDI-IMS
TuPo03	**K. J. Rudzinski, D. Staszek, M. Asztemborska, M. Cieślak, J. Raczko and R. Szmigielski**	Troubleshooting sample preparation of *dendrolimus pini* sex pheromone for ms analysis
TuPo04	**D. Staszek, K. J. Rudzinski, M. Asztemborska, M. Cieślak, J. Raczko and R. Szmigielski**	Analysis of insect signalling using ion-trap mass spectrometry a case of *dendrolimus pini*
TuPo05	**P. Wach-Panfiłow, G. Spólnik, K. J. Rudziński, M. Shalamzari, W. Maenhaut, M. Claeys and R. Szmigielski**	Exploration of aqueous organosulfates from isoprene by LC/MS/MS

TuPo06	**K. Lech, D. Dąbrowski and M. Jarosz**	Identification of tannins by HPLC-UV-VIS-ESI MS in plant dyestuffs
TuPo07	**A. Dias, M. Conceição Oliveira**	Characterization of Synthetic Dyes with Historical Interest by High Performance Liquid Chromatography Coupled with Diode Array Detector and High Resolution/Tandem Mass Spectrometry (HPLC-DAD-MS/MS)
TuPo08	**F. D. Yudin and M. Culea**	Accumulation and estimation of heavy metals in water, sediment and fish from Danube River, Romania
TuPo09	**K. Syslová, M. Mikoška, J. Vondroušová, M. Haluzík and P. Kačer**	Molecular insight into the postoperative state of diabetic patients
TuPo10	**J. Vondroušová, K.Syslová, M.Mikoška, H. Tejkalová, J. Horáček, P. Kačer**	Method development of monitoring of tryptophan and its metabolites in brain tissue of individuals with immune dysregulation and latent toxoplasmosis
TuPo11	**M. Mikoska, K. Syslova, G. Tsenov, P. Kacer**	Molecular approach to focal cerebral ischemia
TuPo12	**A.M. Iordache, C. Voica, I. E. Geana, S. N. Suvar, R. E. Ionete, M. Culea**	Assessment of heavy metals in cosmetic products for health impact
TuPo13	**S. Şuvar, E. Horj, D. Florescu, A. Iordache, D. Cocan and M. Culea**	Analysis of plasma and meat fatty acids in trout by GC/MS
TuPo14	**J. Beinhauer, A. Hanzalová, P. Horčička, M. Šebela**	Intact spore MALDI-TOF mass spectrometry of puccinia pathogenic fungi
TuPo15	**T. Pluháček, M. Petřík, D. Milde and V. Havlíček**	Bioimaging of ferriforms of siderophores in lung tissues by LA-ICP-MS
TuPo16	**E. Tóth, K. Vékey, O. Ozohanics, I. Dominczyk, P. Widlak, L. Drahos**	Changes of protein glycosylation in the course of radiotherapy
TuPo17	**D. Luptáková, L. Krásný, I. Juránek and V. Havlíček**	Lipid profile changes in hypoxic-ischemic neonatal rat brain identified by imaging mass spectrometry
TuPo18	**L. Hartmanová, I. Lorencová, P. Fryčák, H. Chmelíčková, T. Ingr, V. Havlíček and K. Lemr**	Lateral resolution of desorption nanoelectrospray
TuPo19	**Z. M. Nikolic, B. Nastasijevic, D. Acimovic, T. Brdaric, G. S. Tasic, V. M. Nikolic, M. P. Marceta Kaninski**	Determination of pahs content in concrete and mineral oils using UPLC/MS method
TuPo20	**A. Puszko, B. Wileńska, A. Misicka**	General profile of peptides and lipids in rat kidney

TuPo21	**M. Kupiec, K. Pawlak**	Characterisation of hydrolysis products of auranofin by ESI-MS
TuPo22	**M. Kupiec, K. Pawlak**	Probing of auranofin metabolism by single compound EC-MS
TuPo23	**M. Kupiec, K. Pawlak**	The influence of electrochemical reaction chamber on stability of gold(III) complex
TuPo24	**M. Waliczek, M. Kijewska, P. Stefanowicz, Z. Szewczuk**	Mass spectrometry as a research tool for analysis of modified peptides containing oxidized threonine
TuPo25	**M. Szultka-Mlynska, B. Buszewski**	Application of SPME-LC/MS^n for metabolomics drug monitoring in biological samples
TuPo26	**H. Płóciennik, M. Modzel, P. Stefanowicz**	A new method of oxygen-18 labeling of amino acids or peptides in quantinative proteomics
TuPo27	**B. Wileńska, A. Tomczyszyn, A. Szurpnicka and A. Misicka**	The influence of platinum(II) ion on fragmentation mechanism of peptides complexed with platinum(II)
TuPo28	**P. Świder, K. Mulewski, B. Wileńska, W. Danikiewicz**	Radical anions formation in the gas phase addition-elimination reaction of phenide ions with aldehydes
TuPo29	**K. Brama and K. Pawlak**	Analysis of nicotianamine complexes by means of hilic ESI MS/MS
TuPo30	**L. Ceraulo, D. Bongiorno, V. Turco Liveri and S. Indelicato**	Ion mobility mass spectrometry of dimethyl ephedrinium bromide (DMEB) supramolecular aggregates
TuPo31	**A. Wojakowska, Ł. Marczak, M. Chekan, K. Polanski, P. Widlak, M. Pietrowska**	GC/MS profiling of FFPE tissues for screening potential metabolomic biomarkers of different thyroid cancer types
TuPo32	**M. Pietrowska, J. Polańska, A. Wojakowska, K. Jelonek, M. Kalinowska-Herok, I. Domińczyk, E. Chawińska, T. Rutkowski, K. Składowski, L. Miszczyk, P. Widłak**	Whole body response to radiation in head and neck and prostate cancer patient; the serum proteome comparative analysis
TuPo33	**C. Decroo, M. Demeyer, G. Caulier, J. De Winter, P. Flammang, J. Cornil and P. Gerbaux**	Ion mobility as a promising tool to definitively access the molecular structure of saponins
TuPo34	**G. Spólnik, M. Kania, M. Olejnik, M. Sojka, K. Nestorowicz, A. Sobczak, L. Lipiński, A. Dziembowski, W. Danikiewicz**	Differences in soil extraction effectiveness for DDT and its degradants
TuPo35	**G. Spólnik, M. Kania, M. Olejnik, M. Sojka, K. Nestorowicz, A. Sobczak, L. Lipiński, A. Dziembowski, W. Danikiewicz**	Pesticides degradation: storage in specified condition in opposite to presence in soil

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MONDAY ORALS

CHARACTERIZATION OF SECONDARY ORGANIC AEROSOL FROM GREEN LEAF VOLATILES AT THE MOLECULAR LEVEL USING MASS SPECTROMETRIC APPROACHES

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During the past decade considerable progress has been made in our understanding of the fate of biogenic volatile organic compounds (BVOCs), including isoprene and monoterpenes (i.e. α-pinene, β-pinene, *d*-limonene, and Δ^3-carene), which are emitted in large amounts from the vegetation into the atmosphere (Nozière et al., 2015). Secondary organic aerosol (SOA) formation from the photo-oxidation (hydroxyl radical-initiated reactions) or ozonolysis of these BVOCs has been studied under atmospherically relevant conditions. However, relatively little is known about SOA formation from green leaf volatiles, which are emitted from plants when they are wounded or attacked by insects.

A group of SOA marker compounds that has received particular attention are organosulfates, involving reactions of epoxy- or double bond-containing compounds or intermediates with sulfuric acid, which is mainly from anthropogenic origin. Polar organosulfates are of climatic relevance because they are hydrophilic and increase the capacity of aerosols to serve as cloud condensation nuclei.

In this study, we have examined organosulfate formation from 2-*E*-hexenal and 3-*Z*-hexenal, and their photolysis product, the C_5 unsaturated aldehyde 2-*E*-pentenal. Use was made of liquid chromatography/negative ion electrospray ionization mass spectrometry [LC/(–)ESI-MS], high-resolution MS, and detailed interpretation of MS data. The structural characterization of polar organosulfates with MWs 230, 226, 214 and 170, having abundances in ambient fine aerosol comparable to those of the major isoprene-related MW 216 organosulfates, as well as their formation pathways, will be addressed.

Ref.: Nozière, B., M. Kalberer, M. Claeys, et al., The molecular identification of organic compounds in the atmosphere: State of the art and challenges, Chem. Rev., 2015; doi: 10.1021/cr5003485.

ELECTROSPRAY TANDEM MASS SPECTROMETRY OF AQUEOUS-PHASE ISOPRENE-DERIVED SECONDARY ORGANIC AEROSOLS

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Atmospheric aerosols are a complex mixture of solid and/or liquid particles of minute diameters ($d < 100$ μm) suspended in the air. Aerosols significantly influence the Earth's climate and deteriorate the human health and well-being. The adverse health effect of aerosols results from the inhalation of fine and ultrafine particles of poorly recognized chemical properties which contributes to the development of asthma and cardiovascular diseases. According to WHO, the air pollution reduces by a year or more the life expectancy for people living in European cities [1]. Despite the continuous multidisciplinary research, the understanding of the formation and transformation of atmospheric aerosol is still a great scientific *terra incognita* [2].

One of relevant pathways of the atmospheric aerosol formation is the oxidation of volatile organic compounds emitted by living vegetation (i.e., isoprene and its oxygenates), by atmospheric oxidants (i.e., ozone, $HO^{\cdot}$, $SO_4^{\cdot-}$ and $NO_3^{\cdot}$), followed by further processing. These aerosols are denoted as secondary organic aerosol (SOA). In the current study we have evaluated the role of methacrylic acid, $CH_2=CH(CH_3)COOH$, in the formation of SOA components in the laboratory framework. Methacrylic acid is one of the relevant unsaturated C-4 volatiles that exists in the atmosphere at a concentration level of tens of ppb as a consequence of the isoprene ozonolysis. On the other hand, methacrylic acid bears a conjugated double bond system, which renders the compound to be a relevant source of secondary organic aerosols, so far unrecognized.

In this work we applied a self-designed simulation chamber to evaluate the fate of methacrylic acid in diluted aqueous solutions containing sulfoxy radicals that mimic in-cloud atmospheric conditions. In the paper, we will present the selected results of the chemical characterization of the unknown SOA products obtained from SO_4^{-} initiated transformations of methacrylic acid using a reversed-phase liquid chromatography electrospray negative ion tandem mass spectrometry.

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INFORMATION CONTENT OF TANDEM MASS SPECTRA

Fanni Laura Bazsó, Péter Janzsó, Oliver Ozohanics, László Drahos

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Practical applications of mass spectrometry in proteomics and in the biomedical field show increasing importance. Nevertheless, there is little effort directed to understand basic mass spectrometric processes.

During a typical proteomics experiment many tandem mass spectra are recorded. It is well known that collision energy has a crucial effect on the quality of tandem mass spectra. To obtain the most information an optimal energy has to be used: If the collision energy is too low the molecular ion, if it is too high just high energy fragments will be observed.

We have developed novel mathematical expressions to characterize the “purity” and “information content” of a tandem mass spectra. “Purity” in this context means the fraction of ions, which can be accounted for. “Information content” proportional to the number of peaks that can be explained in a spectrum. For example, at low energy the spectra are “pure” (we can explain all ions), but the information content is low (there are few fragments, and low sequence coverage can be gained). We have developed various concepts and formulas to characterize spectra in this way. This provides a novel possibility to efficiently optimize mass spectrometry conditions to suit diverse applications. During our work collision energy dependent tandem mass spectra of model peptides have been measured and these expressions were calculated in each spectra. The calculated values were compared with the mascot scores and the survival yields, and will be discussed in detail in the presentation.

Acknowledgement

This work has been supported by the Hungarian Scientific Research fund (grant No. OTKA K 109006).

ENERGY-RESOLVED TANDEM MASS SPECTROMETRY FOR THE STRUCTURAL AND QUANTITATIVE ANALYSIS OF ISOBARIC/ISOMERIC MIXTURES

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There is a growing demand on mass spectrometric techniques for high-throughput analysis, to perform more reliably and provide more information on increasingly complex samples: analysis of compounds in biological samples, in the field of petroleomics, to study isomers or simply for separating unresolved chromatographic peaks. Identification and quantification of isobars remain challenging tasks for mass spectroscopists, but may become essential to perform the structural analysis and assess the purity of a compound of interest.

CID tandem MS experiments were performed with Ar collision gas on a Micromass/Waters Quattro II QqQ instrument using direct injection ESI for three types of isobaric/isomeric samples: 1) synthetic polymers, poly(tetrahydrofuran) and poly(lactic acid), 2) linear and cyclic topoisomers (P_L-LA) and, 3) linear and cyclic peptide topoisomers.

Energy-resolved CID tandem MS experiments enabled to unequivocally separate and quantify compounds from isobaric/isomeric mixtures of two products. The Survival Yield curves[1] were obtained and, are shown to consist in a linear combination of the curves corresponding to the two components separately[2]. For such mixtures, a plateau appears on the diagram in lieu of the continuous decrease expected, the vertical position of which relates linearly to the relative concentration of the two compounds and was used for quantification using the standard addition method. While typical analytical methods, including NMR, SEC and MALDI-MS, were unable to identify linear residues in a sample consisting of cyclized (P_L-LA) obtained by click-chemistry cyclization, our approach allowed for $(2,1 \pm 0,9)$ % to be detected[3]. The same approach was successfully applied to cyclic peptides obtained by 1,3-Huisgen cycloaddition from functionalized linear compounds at different masses. The proposed methodology for quantification has been tested and validated using chemometrics approach (linear dynamic range, robustness, sensitivity, detection limit ...) using commercially available PTHF and PLA synthetic polymers.

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PEPTIDES LABELED WITH PYRIDINIUM SALTS FOR SENSITIVE SEQUENCING BY ELECTROSPRAY TANDEM MASS SPECTROMETRY

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The insufficient ionization efficiency of some peptides and the resulting limited sensitivity is one of the main problems during analysis of trace amount of peptides by mass spedctrometry. Therefore, the development application of novel ionization enhancers is the main way to overcome this problem [1]. Recently, we developed an efficient method of synthesis of peptide conjugates containing various *N,N,N*-trialkylglycine moieties [2]. We applied them as ionization enhancers for analysis of trace amount of peptides using nano-LC-ESI-MRM technique [3].

We developed new ionization enhancers based on well-known 2,4,6-triphenylpyridinium and 2,4,6-trimethylpyridinium salts. Using of inexpensive and commercially available pyrylium salt allows the derivatization of primary amino groups, especially these sterically unhindered, such as glycine, alanine or ε-amine group of lysine.

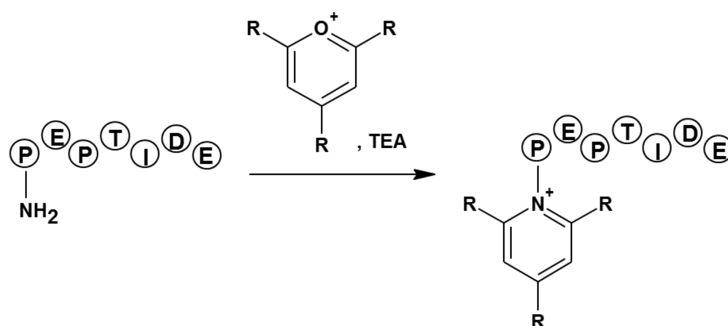

Figure 1. Synthesis of the pyridinium modified peptides (R- methyl or phenyl).

The 2,4,6-triphenylpyridinium or 2,4,6-trimethylpyridinium modified peptides generate an abundant 2,4,6-triphenylpyridinium fragment from the peptide conjugate in MS/MS experiments. This announces a promising reporter ion for the multiple reaction monitoring (MRM) analysis. The fixed positive charge of the pyridinium group enhances the ionization efficiency, moreover pyridinium modified peptides provide full sequence coverage during fragmentation experiment. Analysis of such spectra is facilitated due to the presence of series *b* ions only (Figure 2). The other advantages are the simplicity of derivatization of peptides and the possibility of formation of the pyridinium salt both in the solid-phase peptide synthesis (SPPS) and in the solution synthesis. We presume that the application of such labeling may revolutionize comparative proteomics, leading to the development of new biomarkers based on proteins of low abundance.

Acknowledgments:

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DE NOVO IDENTIFICATION OF NONRIBOSOMAL PEPTIDES FROM ACCURATE PRODUCT ION MASS SPECTRA

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Nonribosomal peptides have a wide range of use in many biological applications and in pharmacology. Their identification by tandem mass spectrometry is a challenging task due to their complex structures. A new stand-alone, cross-platform and open-source *de novo* identification engine CycloBranch was developed and successfully applied in identification or detailed characterization of eleven linear, cyclic, branched and branch-cyclic peptides. The application is based on an annotated database of almost 300 nonribosomal building blocks in contrast to commonly used *de novo* tools using a restricted database of proteinogenic amino acids. CycloBranch has a graphical and user-friendly interface and it can be downloaded for free from [1], where the User's manual and video tutorials can be found.

Acknowledgements

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THE EMBRYOME: MOLECULES IN SPACE AND TIME DURING THE EARLY STAGE DEVELOPMENT

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Human infertility is a global problem with significant social and economic impact. Successful pregnancy is a complex process that comprises unique events, including fertilization, implantation, decidualization, placentation and finally birth. Nowadays, the assisted reproduction technologies (ART) have provided remarkable impact for successful pregnancy after *in-vitro* fertilization. However, these methods are associated with relatively low clinical pregnancy rate of approx. 30% per transfer.

In this multidisciplinary study, multimodal imaging techniques, such as matrix-assisted laser desorption ionization time-of-flight imaging mass spectrometry, fluorescent confocal microscopy and histochemistry were used for *in-vitro* molecular analyses of the cryosections of CD-1 mice uteri. The lipids were identified by liquid chromatography coupled ultra high-resolution tandem mass spectrometry.

The 51 uteri samples (technical triplicates from each group) were collected from eostrus cycle, pseudopregnant and pregnant animals (natural and transferred embryos) at embryonic day of 4.5, 6.5, 8.5, 10.5, 12.5, 17.5, respectively.

Our findings showed significant changes of phospholipid spatiotemporal distribution between the sample cohorts, the phosphatidylcholines and phosphatidylethanolamines as well as the sphingomyelins altered by eostrus cycle, age of pregnancy. Surprisingly, remarkable molecular differences (phospholipids, ceramide, diacylglycerol) were detected between the identical stage transferred and natural (non-transferred) embryos.

The main limitation of the present study is that the mass spectrometry imaging is an invasive technique, so it could be used on animal models. However, the mouse models have contributed greatly to our understanding of the physiological and molecular events necessary for the process early stage embryogenesis.

ION MOBILITY MASS SPECTROMETRY, FROM RESEARCH TO ROUTINE

Matthew Kennedy, Bogdan Czerski

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The combination of ion mobility (IM) and time of flight (TOF) mass spectrometry (MS) was first presented by Edelson and Young in 1967. Although the benefits of combining these techniques were clearly understood and substantial, the technical difficulties delayed the commercialisation of an IMS-TOF for almost 50 years, until the launch of the Synapt™ in 2006.

In addition to summarising the last decade of IM-MS development, this presentation will describe the reasoning behind building the first R&D prototype IMS TOF in 2001 and the future hopes Waters has for the combination of Ion Mobility and TOF.

It is the personal opinion of the presenter, that ion mobility provides five key advantages for mass spectrometry, as described below. These five topics will be described and depending on interest and time can be elaborated on.

Achieving What No MS Can
- Isobaric Separation

Enhanced MS Performance:
- Improving System Peak Capacity

Additional Data
- Size & Shape Characterisation (CCS
- Class Classification

Enhanced Efficiency
- Linked Scans (Sensitivity, Compound Class etc)

Enhanced Fragmentation

Finally the presentation will describe one of the benefits ion mobility has brought to the MS community, specifically the discovery of "protomers". Protomers are compounds with multiple sites of protonation or deprotonation that can result in multiple structures and fragmentation patterns for the same compound.

INVESTIGATION OF PROTEIN BEHAVIOUR ON MICROSECOND TIME-SCALES USING DUAL-CHANNEL NANO-ELECTROSPRAY EMITTERS

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Dual-channel nano-electrospray emitters each containing two separated channels running through the length of the emitter have been fabricated from “theta-shaped” borosilicate capillaries[1]. The diameters of the tips of the emitters have to date typically been of the order of a few micrometres. Behaviour of proteins and other biomolecules has been investigated on microsecond time scales using these emitters coupled with Fourier transform ion cyclotron resonance mass spectrometers. Loading of different solutions into the two channels of an emitter opens up the possibility of studying interactions of the solutions within a common Taylor cone[2]. A Taylor cone typically represents a “mixing volume” of the order of femtolitres. An open question is the extent to which observed products of reactions were formed in the Taylor cone, in comparison with the weight of contribution from subsequent events within droplets. Results with vancomycin and the tripeptide KAA each introduced separately into one channel of a dual-channel emitter have evidenced formation of the doubly charged non-covalent complex (vancomycin+ KAA)[2] . Similarly , introducing vancomycin and deuterated vancomycin into the separate channels has demonstrated H/D “scrambling”, implying rupture and formation of covalent bonds during the transition from tip to mass spectrometer. There is evidence of protein folding and unfolding having been induced by introducing a protein through one channel and using the other channel to adjust the pH of the mixed solutions [3,4] . Our aim as described here is develop dual-channel nano-electrospray for ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry as a reliable method for probing rates of protein-ligand binding and rates of protein folding.

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THE APPLICATION OF MASS SPECTROMETRY FOR THE ANALYSIS OF THE CRYPTIC PEPTIDES PRODUCED BY THE ACTION OF IDE ON SOME OF ITS SUBSTRATES

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In recent years, Insulin-Degrading Enzyme (IDE) has drawn attention because of its capability to degrade β-amyloid peptides as well as insulin and it is currently studied as a pharmacological target for Alzheimer's disease. Our group has already reported that IDE activity can be conveniently screened by mass spectrometric methods. Particularly, in this work the unique potentiality of mass spectrometry to study the cryptic peptides generated by the action of IDE on some of its substrates will be described.

A SYNTHESIS OF NEW, BI-LABELLED PEPTIDES FOR QUANTITATIVE PROTEOMICS

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Mass spectrometry is one of the most important analytical methods used in proteomics. It readily provides data concerning the identity of the analysed proteins and can also be used to study the post-translational modifications. However, absolute quantification by mass spectrometry is not straightforward and requires special techniques – usually applying isotopic labelling. Several such strategies have been described.[1]

We propose a new strategy, well suited to quantitative analysis of post-translationally modified proteins, basing on the concept of analytical constructs. They are composed of an isotopomer of a peptide formed during the enzymatic cleavage of the analysed protein, a linker with a cleavage site, and a chromophore moiety. Our experiments have proven that the response factors for peptide – chromophore conjugates do not depend on the peptide sequence. The construct can be quantified basing on an HPLC-Vis measurement, and then added to the sample before the hydrolysis step. Finally, the hydrolysate containing the light peptide from the protein and the heavy one from the construct can be analysed by LC-MS and the concentration of the parent protein can be assessed by comparing the intensities of the MS peaks corresponding to these two peptides.[2]

Acknowledgments:

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BASICITY AND ACIDITY INFLUENCE ON THE STABILIZATION OF THE HYDROGEN BOND AND SALT BRIDGE COMPETITIVE INTERACTIONS IN ANIONIC ARGININE/HEXOSE-PHOSPHATE COMPLEXES

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A lot of non-covalent interactions exists in aqueous solution although salt may be dissociated. In mass spectrometry, it is possible to preserve non-covalent interactions (NCI) and thus, it is an useful tool for studying non-covalent complexes (NCC). In particular, under ESI conditions, ion-ion interactions (e.g. salt bridge SB) can appear in competition with ion-dipole such hydrogen bond (HB) into the produced multi-charged aggregates. In the gas phase, their survival is possible into solvent-free NCC. From relatively small size NCC, more stable HBs mainly occur between similar functional sites characterized by close basicities for positive or acidities for negative ions. This contrasts with SB which requires a very acid site on one partner in interaction with a second patner containing both an very acid and a very basic sites. This means that structure of one of two partners must be within zwitterion ZW form and the second as protonated or deprotonated for cationic or anionic complexes, respectively. Recently, we demonstrated the SB/HB coexistence into solvent-free deprotonated NCC constituted by both the fructose-phosphate isomer and arginine partners from desolvation of multi-charged aggregates occurring under different “in-source” conditions. CID spectra were used to distinguish anionic complexes stabilized by either HB or SB interactions. Indeed, they dissociate *via* diagnostic pathways allowing their respective attribution.

In order to rationalize the experimental results, the role of the site basicity and acidity, as well as the entropic effects on (i) stabilization of the HB/SB forms according to the desolvation, and (ii) orientation of fragmentations for each non-covalent species will be investigated. To highlight the role of thermochemical parameters, the behavior of sodiated deprotonated forms will be explored through their respective stability and their diagnostic dissociations. The structures of the CNC will be considered depending on whether complexes HB or SB from either deprotonated molecules or sodiated plus doubly deprotonated NCC. We will show why these forms are inconvertible. From all these results, a model interpreting why SB species do not appear in low energy desolvation while they are observed mainly in high-energy and vice versa for HB forms. A energetic pathway will be proposed to explained the HB and SB form inconvertibility.

ESI-MS^N STUDY OF THE INTERACTION PRODUCTS OF A CYTOTOXIC PHOSPHINO COPPER(I) COMPLEX WITH METHIONINE-RICH PEPTIDES

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The cytotoxic activity of the copper(I) complex $[Cu(thp)_4][PF_6]$ (CP) (thp = tris-hydroxymethyl phosphine) is correlated with its high accumulation in cancer cells. Human Copper transporter 1 (hCtr1) has been described as the main trans-membrane protein involved in cellular trafficking of physiological copper [1]. Methionine-rich peptide sequences incorporated in the extracellular domain of hCtr1 play a key role in the cellular internalization of copper. In this study we have investigated the interaction of CP with model peptides that mimic the extracellular domain of hCtr1. The interaction of CP with methionine-rich and methionine-free model peptides has been investigated in electrospray ionization mass spectrometry and the interaction products have been characterized by multiple collisional experiments, using an ion trap mass instrument.

The interaction of CP with the following model peptides, Ac-MMMMPMTFK-NH_2 (P1) and Ac-MGMSYMDSK-NH_2 (P2), shows that the native copper complex, after sequential loss of phosphines [2], induces the formation of $[Cu(P1)(thp)]^+$ and $[Cu(P1/P2)]^+$ adducts reasonably by inclusion of the Cu(I) ion in the peptide framework. Collisionally induced fragmentations (MS^n) of $[Cu(P1/P2)]^+$ give evidence that the metal is coordinated by the thioether-S of two adjacent methionine residues. Interaction of the same peptides with the isostructural complex $[Ag(thp)_4]^+$ or $AgNO_3$ yields similar experimental evidences, leading to $[Ag(P1/P2)]^+$.

Methionine sequences incorporated in the model peptides are crucial for the recruitment of copper from CP. Such a metal-peptide interaction does not take place when methionine-free Ac-NleGNleSYNleDSK-NH_2 (P3) is utilized. A mechanism for tumor cell internalization of CP involving: *i)* chemically driven sequential loss of phosphines from the native tetrahedral complex [2], followed by *ii)* transfer of Cu(I) to the methionine-rich sequences typical of the hCtr1 transporter, is proposed.

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FUNDAMENTALS AND ADVANCES OF ORBITRAP MASS SPECTROMETRY – APPLICATIONS AND CURRENT TRENDS IN ANALYSIS

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Recent developments in the field of mass spectrometry fulfill the still growing demands of modern analytical chemistry. High resolution, mass accuracy and sensitivity are essential needs in numerous areas of research as well as in routine laboratory analysis. The Orbitrap-based mass spectrometers have established themselves firmly in the field of omic sciences e.g. proteomics, metabolomics, glycomics and lipidomics. What is more, benefits of the Orbitrap technology are spreading out on different fields of analytical chemistry such as bioanalysis, anti-doping, environmental and food safety (pesticides, mycotoxins) or forensics and toxicology (drug residues and their metabolites). Mass spectrometer with Orbitrap mass analyzer is a tool for targeted quantitation as well as for discovery of unknowns. Current presentation shows the principle of operation of Orbitrap analyzer and discuss application areas [1] where Orbitrap analyzers represent the state-of-the-art solution.

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TUESDAY ORALS

STUDYING ORGANIC REACTIONS MECHANISM BY ESI-MS: THE CASE OF THE UGI AND UGI-SMILES REACTIONS

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During the last few years, ESI-MS has widespread greatly as an effective technique for mechanistic studies of organic reactions. In particular the so-called “ion-fishing” methodology offers the opportunity to investigate in the gas-phase key-species with short lifetimes at very low concentration.

The Ugi reaction, one of the most prominent multicomponent reaction in organic synthesis, still is under debate as far as its reaction mechanism is concerned. The reaction sequence involves participation of an aldehyde, a primary amine, an isocyanide and, in its original formulation, a carboxylic acid leading finally to α-aminoacyl amide derivatives. The use of difunctional reagents, or just the replacement of the last component with other nucleophiles, multiplies the number of accessible scaffolds. Among these, the Ugi-Smiles variation is characterized by the employment of electron-deficient phenols instead of the carboxylic acid. To date, two mechanistic proposals are the most accredited ones for this multicomponent reaction: i) the original pathway introduced by Ugi himself; ii) a more recent proposal which points to a delayed isocyanide insertion. In a recent study, both the Ugi and the Ugi-Smiles reaction mechanism are studied in detail under the theoretical point of view [1]. A few months ago an ESI-MS investigation of the Ugi reaction also appeared, which reports on the use of charged tagged reagents to catch intermediates for sensitising them to ESI-MS detection [2]. Such tagged reagents, however, might introduce a charged bias in the reaction mechanism itself.

Following our general interest in the applications of mass spectrometry in the field of organic chemistry, we report here on our recent results [3] on the study of both the Ugi and the Ugi-Smiles reactions by ESI-MS by way of a different strategy, which does not require charge-tagged reagents. The Ugi and Ugi Smiles reactions were carried out using a few accurately selected reaction conditions which allowed to “fish out” and characterise all the intermediates by ESI-MS/MS. The reaction mixtures were directly infused into a Q-Tof Mass Spectrometer (Xevo G2 QTof; Waters) equipped with the ESI source. Strong evidences for the Ugi and Ugi-Smiles reaction mechanism have been collected. Crucial intermediates have been intercepted and structurally characterized by ESI-MS and MS/MS. All the data support strongly the original hypothesis by Ugi of a via nitrilium-ion mechanism. Remarkably also, all our data are in perfect agreement with the theoretical calculations [1].

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INTERACTION OF METAL CATIONS WITH ALKYLNITRILES, ALKYLAMINES AND ALKYLALCOHOLS IN THE GAS-PHASE : SOLVATION OF METAL IONS BY THE HYDROCARBON CHAIN

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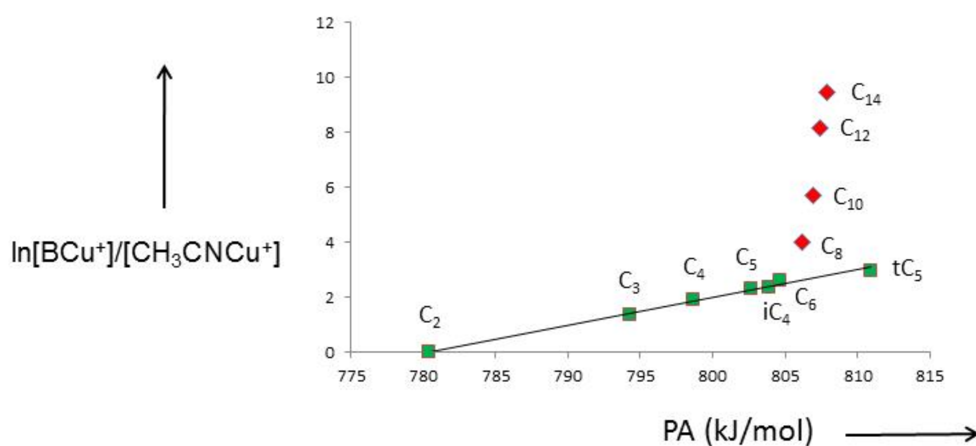
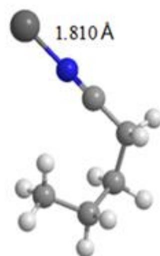
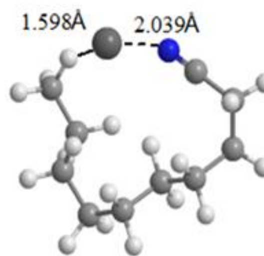
Relative affinity measurements of monovalent metal ions (= Li^+, Na^+, Cu^+ and Ag^+) towards aliphatic nitriles, amines and alcohols have been performed using the kinetic method by dissociation of metal bound dimer ions. It is found, particularly for Cu^+ and Ag^+, that the affinity towards long aliphatic chains (> C_6) is markedly enhanced. This is attributed to a bidentate interaction of the metal ion with the nitrile, amine or alcohol moiety and the aliphatic chain. Theoretical calculations on the copper complexes of the nitriles show that these bidentate structures enjoy about 30% greater copper ion affinities compared to their linear counterparts. For the nitriles, such aliphatic interactions also play a major role in the dissociation chemistry of copper bound tetramers of the kind $(RC{\equiv}N)_4Cu^{2+\bullet}$ where the long aliphatic chain R curls around the copper ion facilitating electron transfer or a redox reaction to produce $(RC{\equiv}N)_2Cu^+ + RC{\equiv}N^{+\bullet} + RC{\equiv}N$.







FRAGMENTATION OF PROTONATED 2-(2-PHENYLETHYL)CHROMONES: THE DIAGNOSTIC ROLE OF ION/NEUTRAL COMPLEXES

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An (+)-ESI-CID mass spectrometric study on the fragmentation of the $[M + H]^+$ ions of 2-(2-phenylethyl)chromone and a set of nine hydroxyl- and/or methoxy-substituted derivatives, isolated from agarwood,[1] has revealed a highly prominent fragmentation channel, namely the loss of benzoquinonemethanes or benzaldehydes as a structurally diagnostic feature. 2-(2-Phenylethyl)chromones that bear a hydroxyl group at the *para*- (4'-), *ortho*- (2'-) or/and benzylic (α-) position of the phenylethyl residue undergo such an elimination, whereas all other derivatives, including those that bear a *meta*- (3'-) hydroxyl group, do not. The intermediacy of ion/neutral complexes (INCs) is invoked to explain this fragmentation, which involves the remarkable intra-complex proton or hydrogen atom transfer from the remote 4'-OH (or the 2'- or α-OH) functionalities. This remarkable fragmentation behaviour will be discussed in a broader context of ion/neutral complex-mediated fragmentations.[2]

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GAS-PHASE REACTIONS OF METHYL THIOCYANATE AND DIMETHYL DISULFIDE WITH CARBANIONS

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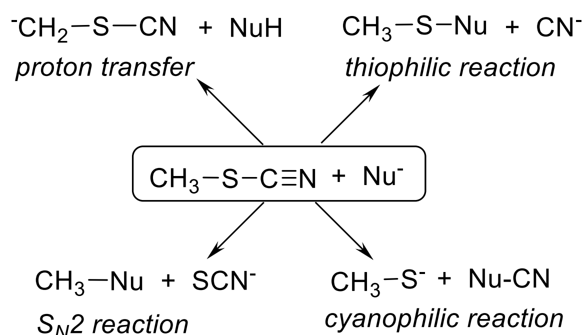
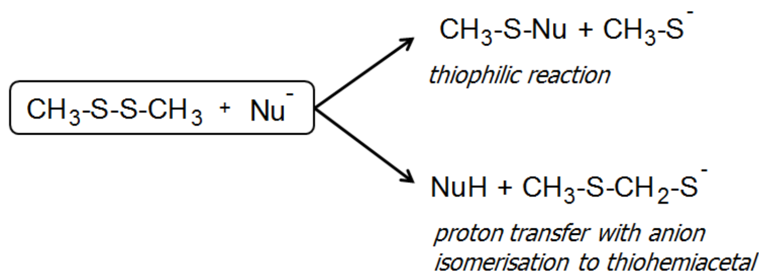
The gas-phase reactions of aliphatic and aromatic carbanions with methyl thiocyanate and dimethyl disulfide have been examined for the first time. The analysis of the spectra shows variety of product ions formed via different reaction mechanisms, depending on the structure and proton affinity of the carbanion.

In case of reactions of carbanions with MeSCN considered pathways are: S_N2 nucleophilic substitution (SCN^- product ion, *m/z* 58), cyanophilic reaction (CH_3S^-, *m/z* 47), thiophilic reaction (CN^-, *m/z* 26) and proton transfer($^-CH_2SCN$, *m/z* 72). Secondary products of the cyanophilic reaction are also visible in the spectra. Proposed reaction pathways are supported by DFT calculations. [1]



For the reactions of carbanions with Me_2S_2 preliminary data suggest that main reaction pathways are thiophilic reaction (CH_3S^-, *m/z* 47) and proton transfer (*m/z* 93 – DFT calculations suggest that this product ion peak corresponds to $CH_3S\text{-}CH_2S^-$).



Further analysis of the spectra shows that thiophilic product undergoes secondary reactions – S_N2 and proton transfer. Additionally, in the case of aromatic carbanions, substitution of fluorine atom with CH_3S- group was observed. No such substitution occurred for carbanions containing bromine and chlorine atoms.

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CORRECTING LONG-TERM DRIFTS IN HPLC-MS

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It is well known, that MS tuning drifts in time – this is the main reason for regular instrument tuning. This may become a critical issue, when long series of HPLC-MS runs are performed. Electrospray, and in particular nano-ESI is particularly sensitive in this respect. One possible cause might be the continuous changes at the tip of the capillary due to e.g. electrochemical processes or partial blocking of the spray.

We have studied this phenomenon in the course of proteomics experiments. Typical conditions include a series of 1-2 hour long HPLC (or nano-HPLC) runs lasting for several days. We found (on various instruments and laboratories) that the measured abundance ratio of various peptides changes significantly under such conditions; this change is mostly in the 10-50% range. Note that this is a systematic change, random variations of peak abundances is a different issue altogether. We have also found that different signals (peptide abundances) vary differently; on peptide abundance may decrease, another might increase in the series of HPLC-MS runs. This means, that normalizing peptide abundances to the sum of all peak abundances (‘total ion current’) is useful (removes part of the error), but does not solve the problem.

We have run the same sample for several days, and found that the variability of a given peak abundance in time can be well described by a 4th order polynomial function. Taking this into account peak abundances can be corrected using this function; which successfully removes all (or at least most) systematic errors. Repeating the procedure for all components in a mixture the systematic bias due to changing HPLC-MS conditions can be corrected. When a series of plasma samples is studied, it is expedient to make a quality control sample (e.g. by pooling aliquots from all samples), which contains all components to be analyzed. Measuring the QC sample various times during a series of HPLC-MS experiments, the systematic bias can be corrected. In practice, 20-30% of all samples should be quality control.

The developed method can be very well used in practice. In one case (60 HPLC-MS runs, lasting 3-4 days) this correction decreased errors in peak abundances from 12% to 4%. Other cases yielded similar results.

IMAGING MASS SPECTROMETRY

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In this presentation, the three most recent imaging mass spectrometry applications from Prague lab will be reported. In the first paper [1] we switched our attention to nanostructure-assisted laser desorption ionization (NALDI) based on silicon nanowires. The absence of matrix in NALDI experiments stimulated us to rigorously evaluate the lateral resolving power which can be achieved with our mass spectrometry imaging equipment. The shape and size of actual spots generated in NALDI mass spectrometry were evaluated by scanning electron microscopy. The lithographic imprints were rugged and the achieved lateral resolution was defined by laser incident beam focus exclusively. In NALDI IMS, we demonstrated a routine 20 µm lateral resolution and noticed a decrease of ion yield, when laser focus was increased from 20 to 50 µm at low laser intensities. The effect was rationalized by scanning electron microscopy imaging as laser beam defocusing. In the second application [2], our efforts were directed to investigate the lipid distribution in the kidney of the murine model of Fabry disease. The analysis of the dynamic range and relative signal intensities of the Gb3 isoforms by MALDI mass spectrometry imaging provided similar trends to that of the FIA-ESI-MS/MS analysis. Whereas the Gb3 increase in the FKO renal tissue was 34-fold greater than that in the WT mouse in a calibrated FIA experiment, a similar increase was observed in MSI. As expected, the MSI also revealed a 3-fold increase of the ceramide dihexoside fraction primarily due to the Ga2 accumulation in the FKO kidney compared to that in the WT mouse. In the final application [3] the eye lenses of three human donors were studied with respect to lipid lateral distribution by matrix assisted laser desorption imaging mass spectrometry. Aging process will be discussed in context of the number of lipids detected by exact mass measurements as well as the hydrolytic products derived from sphingomyelins. This is the first study showing the distribution of lysolipids as potential aging biomarkers in human lens tissue. Variable composition was observed in nuclear, barrier or cortex regions of the lens samples.

Acknowledgements

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MASS SPECTROMETRY “ON THE TRACK” OF METALLODRUGS’ METABOLISM

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Metallodrugs are increasingly important in the search for more effective anticancer remedies. However, cancer still causes the death of more than 20.000 people a year, since many widespread tumors are not (or poorly) curable with the established platinum (as well as organic) drugs. Therefore, anticancer metallodrug research needs to be intensified and, in particular, focused on a better understanding of the mode-of-action for developmental non-platinum tumor-inhibiting compounds. Metal-ligand compounds are increasingly important in the search for more effective anticancer weapons. As hybrids of inorganic and organic components, metal complexes tend to assemble the advantages of inorganic and organic drugs, such as treating a broad range of tumors and a selective mode of action, respectively. A promising examples, indazolium trans-[tetrachlorobis(1H-indazole)ruthenate(III)] and tris(8-quinolinolato)gallium(III), auranofin (2,3,4,6-tetra-O-acetyl-1-thio-b-D-glucopyranosato-S-(triethyl-phosphine) gold(I)) or Au(III)bipy^{c} ([(bipy^{dmb}-H)Au(OH)][PF_6] (where bipy^{dmb}-H = deprotonated 6-(1,1-dimethylbenzyl)-2,2′-bipyridine)) are now appreciably progressing in clinical studies, with the outcome of few side effects and evidence of clinical activity in the case of carcinoma cells. In order to make its clinical development more straightforward, further endeavors are expected toward establishing the drug mechanism.

In this regard, the mechanism of drug transport to cell and its metabolism is of primary interest. The role of serum transport proteins in drug delivery to cytosol should be clarified due to the major influence on the bioavailability of the drug. The lack of knowledge on these issues holds back the clinical development of metal complexes, making it tremendously long and expensive. It is of special importance to facilitate the identification and selection of the most efficient, ‘hit’ compounds among novel metal coordination compounds with proven cytotoxic activity, and makes real the synthesis of specific target designed new metal-based drug candidates.

Molecule specific mass spectrometry (ESI/MALDI MS) and isotopic specific mass spectrometry (ICP MS) coupled with electrochemical reaction chamber and different separation techniques such as capillary electrophoresis and high performance liquid chromatography can be considered as a most suitable approach for kinetic and thermodynamic studies of metallodrugs. Moreover, the strategy to study the metallodrugs’ metabolism will be discussed with special emphasis on sample preparation procedures. Advantages and limitations of each technique will be discussed on the basis of the results and experience obtained during studies of transport mechanism of platinum, ruthenium, gallium and gold complexes. New challenging problems will be also reported.

The part of the presented results was obtained in frame of the project financed by the National Science Centre allocated on the basis of a decision DEC number 2013/09/B/ST4/00961

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In my presentation I would like to briefly present SCIEX state of the art portfolio, including unique QTRAP® technology, SelexION™ - device which makes ion mobility spectrometry available for routine analysis, high resolution TripleTOF® series with innovative STWATH acquisition mode and the world's only dedicated splitless microflow UHPL - MicroLC 200 Plus.

SUPRAMOLECULAR MASS SPECTROMETRY: ASSOCIATION OF MS METHODS TO COMPUTATIONAL CHEMISTRY TO ACCESS, AT A MOLECULAR LEVEL, SYSTEMS RELEVANT TO HOST-GUEST CHEMISTRY

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Introduction Host-guest chemistry represents one of the major topics of Supramolecular Chemistry and concerns the design, the synthesis and the characterization of selective/specific receptors able to strongly entangle target molecules within dedicated cavities. Perfect associations between the host and the guest partners rely on the complementarities between their topologies and functionalities. Numerous spectroscopic methods are developed to investigate those non covalent associations with particular interests on the measurement of binding constants and the determination of the structure of the association. NMR and UV-vis spectroscopies are often used to investigate such systems. Nowadays, mass spectrometry has been demonstrated to be a valuable and elegant way of studying non covalent associations extracted from the condensed phase to gas phase by means of a soft ionization method, such as Electrospray.

Methods In the context of our investigation, the experiments are conducted by using conventional mass spectrometry methods such as ESI-MS and ESI-MSMS (CID). In addition, more sophisticated approaches such as energy-resolved CID, ligand exchange experiments and of course ion mobility mass spectrometry were implemented in our work to obtain an in-depth description at the molecular level of the energetics and structure of the gas phase non covalent associations. Beside the experimental part of the project, it is really important to obtain corresponding theoretical data such as optimized structures, energetics, isomerization and decomposition thresholds and collisional cross sections. This aspect of the project relies on the use of state-of-the-art computational methods such as molecular mechanics and dynamics approaches and DFT calculations.

Results For the present communication, we would like to give an overall overview of the results that were obtained in our laboratory and that are related to host-guest chemistry with different receptors such as a chiral crown ether [ChemEuJ 2008], cucurbiturils, a bitopic cucurbituril that present homotropic allosterim capabilities [chempluschem 2013] and a chiral calixarene. Those examples were selected to demonstrate that the association of high level theoretical chemistry with state-of-the-art MS methods represents a powerful tool for investigating host-guest complexes from structural and energetic points of view. Such studies are important to further design specific receptors or sensors for targeted molecules.

Novel aspects Numerous data are reported dealing with non covalent associations between large biomolecules and their ligands. Fewer studies are related to the host guest chemistry domain of research probably because of the availability of the systems to organic chemists nowadays but also since non specific associations are more likely to appear with small molecules than with bigger.

INFLUENCE OF SINGLE SKIMMER VS. DUAL FUNNEL TRANSFER ON THE APPEARANCE OF ESI-GENERATED LICL CLUSTER / ß-CYCLODEXTRIN INCLUSION COMPLEXES

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β-cyclodextrin (βCD), a circular heptamer of α-D-glucopyranose, forms singly and doubly charged adducts of the type $(\beta CD)(LiCl)_nLi^+$ and $(\beta CD)_2(LiCl)_pLi_2^{2+}$ in electrospray ionization mass spectrometry (ESI-MS) [1, 2]. Insight into their structural composition was gained by analysis of their collision-induced dissociation (CID) mass spectra.

Two different instruments were used for the ESI experiments, a quadrupole ion trap (QIT) incorporating a single skimmer, and a quadrupole time-of-flight (QTOF) mass spectrometer utilizing funnel ion guides.

The conditions the ions experience in the transport region interfacing the ESI source and the mass spectrometer were found to have significant influence on the nature of the observed salt adducts. In the skimmer instrument, i.e. under harsher conditions, individually attached LiCl ion pairs were observed, whereas the dual funnel ion guides of the second instrument allow the detection of previously unknown labile inclusion complexes of $(LiCl)_n$ clusters in βCD.

Both types of attachment could also be found in the less stable doubly charged cyclodextrin dimers. All observed species are probably generated in the ESI process and only the conditions in the transfer region determine whether or not they survive long enough to reach the mass analyzer.

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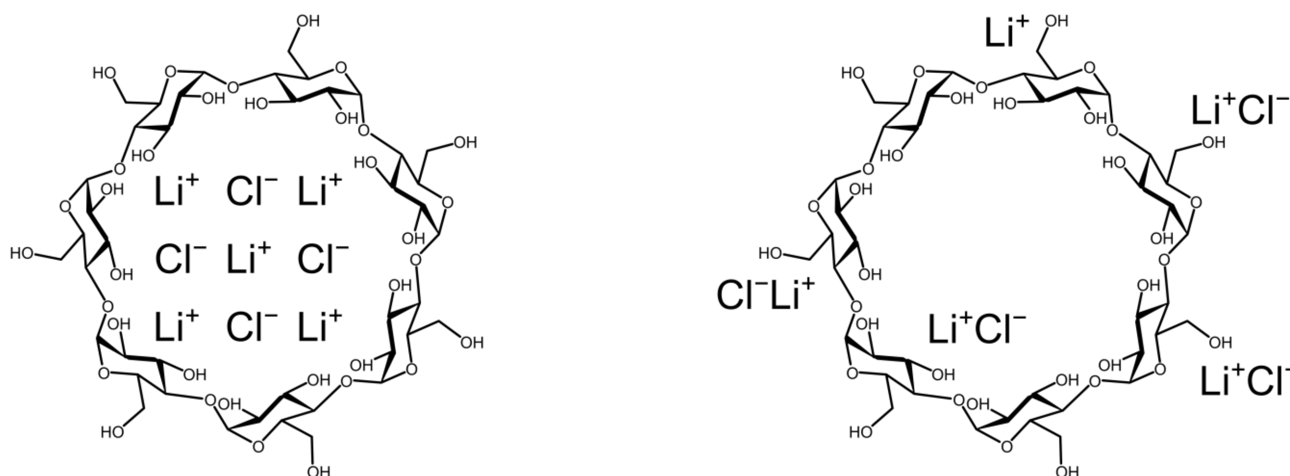

ISOTOPIC FINE STRUCTURE – A PERFECT TOOL FOR UNAMBIGUOUS MOLECULAR FORMULA ELUCIDATION

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Mass spectrometry is a well know technique for molecular formula identification and verification.[1] This technique is based on very accurate mass measurement of the ^{12}C isotope. The $^{12}C/^{13}C$ ratio can additionally be used for molecular formula verification even with low resolution instruments. High resolution instruments can separate the ^{34}S and the $^{13}C_2$ peak of small molecules. Therefore, the number of sulfur atoms in a compound can be directly calculated from the $^{34}S/^{12}C$ ratio. Ultra-high mass resolution of Fourier transform ion cyclotron resonance mass spectrometry with mass resolution above 500.000 gives access to other naturally occurring isotopes like ^{15}N and ^{18}O for unambiguous structure elucidation of nitrogen and oxygen containing compounds.[2] These hetero atoms are quite abundant in biological samples beside sulfur and phosphor. By use of the isotopes ^{13}C, ^{15}N, ^{18}O and ^{34}S the possible molecular formulas can be drastically reduced with respect to the accuracy of the measured mass. In principle the number of oxygen, nitrogen and sulfur atoms of an unknown compound can be directly calculated from the A+1 and A+2 isotopic fine structure pattern if all isotopes in the molecule can be resolved in the mass spectrum. It has been shown that this technique can even be used for counting the number of hetero atoms in peptides when extremely high mass resolution is applied.[3,4] This technique has also been applied to HDX experiments to resolve the ^{2}H mass peaks.[5] Examples of unambiguous determination of metabolites, molecular formula of fragments as well as structure elucidation of metal-organic compounds will be shown.

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COPPER(I)-CATALYZED AZIDE–ALKYNE CYCLOADDITION: AN ESI-MS(/MS) MECHANISTIC INVESTIGATION

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The copper-catalyzed azide–alkyne cycloaddition (CuAAC) can be seen as the example par exellence of the so-called click chemistry. This Cu(I) mediated transformation is the most straightforward strategy for the regiospecific preparation of 1,2,3-triazoles. CuAAC is widely employed in organic synthesis, medicinal chemistry, surface and polymer science, and in bioconjugation strategies. However its mechanism has emerged as particularly complex, being still a matter of debate.

All first-order, second-order third-order rate law with respect to Cu(I) has been experimentally observed, but relate all of them to a common binuclear Cu mechanism. DFT calculations of the reaction pathway showed a more favorable energetic pathway when dinuclear Cu(I) intermediates are considered, as compared to mononuclear intermediates.

However, as only mononuclear copper acetylides and copper triazolides can be isolated, the implication of truly dinuclear Cu(I) intermediates could not be claimed out of any doubt, until a paper by Fokin et al.[1] Indeed, they inferred for the CuAAC the existence of dinuclear copper intermediates by in situ reaction calorimetry measurements, and copper isotope crossover experiments.

Despite these studies and mechanistic data available, however, no direct observation of copper dinuclear CuAAC intermediates has been reported to date.

Therefore we investigated the mechanism of the CuAAC reaction by electrospray ionization mass spectrometry (ESI-MS) using a combination of the neutral reactant approach and the ion- tagging strategy. Under these conditions, for the first time, putative dinuclear copper intermediates were fished out and characterized by ESI(+)-MS/MS. Such non-isolable active dicopper intermediates are consistent with the computational and experimental state of the art for the CuAAC mechanism.

New insight into the CuAAC reaction mechanisms is provided and a catalytic cycle is proposed.[2]

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GAS-PHASE EXPERIMENTS WITH POLYYNES: LASER ACTIVATION AND Ag^+-INDUCED REACTIVITY

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End group-protected linear polyynes of the composition $Tr^*-(C\equiv C)_n-Tr^*$ with Tr* representing the super trityl group ($Tr^* = (tBu_2(C_6H_3))_3C$, n = 2, 4, 6, 8, 10) and $tBu-(C\equiv C)_6-tBu$ with *t*Bu being the tertiary butyl group have been studied by laser desorption ionisation (LDI) time-of-flight (TOF) mass spectrometry. *t*Bu terminated polyyne molecules show considerably higher stability during laser activation than Tr*-end-capped polyynes. A key feature is the abundant formation of oligomeric species upon laser activation. Tandem mass spectrometry reveals strong bonding within the oligomers which indicates cross-linking of the former polyynes. The process is more abundantly occurring and less energy demanding than the laser-induced coalescence of C_{60}. Cross-linking is more efficient with the smaller end group (*t*Bu) and larger oligomers are formed when the chain length of the polyyne increases, both a result of enhanced interaction of the triple bonds in neighbouring chains. The presence of the matrix molecules in matrix-assisted (MA)LDI hinders the polyyne interaction and oligomer formation is markedly reduced.[1] Ag^+ attachment makes the molecules accessible to electrospray ionisation (ESI). Introducing a polyyne/AgTFA solution into the ESI source results in the formation of $[M+Ag]^+$ and $[M+2Ag]^{2+}$ at considerably high intensity and in the formation of the dimeric species $[2M+Ag]^+$ (with M being the neutral polyyne molecule) for most of the polyynes under investigation. For both monomeric species, collisional activation leads to Ag^+-insertion into the sp-carbon chain resulting in the formation of a carbenium ion ($[Tr^*(C_2)_x]^+$) and a silver acetylide ($Tr^*(C_2)_yAg$ and $[Ag+Tr^*(C_2)_yAg]^+$, respectively). Collision-induced dissociation (CID) of the Ag^+-bridged dimer reveals that there is only one notable fragmentation channel: $[2M+Ag]^+ \rightarrow [M+Ag]^+ + M$. Since the formation of hetero dimers (different chain lengths) is observed for mixed compound solutions, the relative silver-ion affinities of the polyynes were investigated applying the kinetic method.

Tr*–≡–Tr*

References

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APPLICATION OF LC/QTOF IN ANALYSIS OF DRUGS, DRUGS CANDIDATES AND NEW PSYCHOACTIVE SUBSTANCES

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Liquid chromatography coupled to high resolution mass spectrometry is becoming gradually a primary analytical method for pharmaceutical, environmental, veterinary, forensic and toxicological applications. It offers high sensitivity and selectivity as well as flexibility and versatility. The development and constant improvement of accurate mass spectrometers including LC/QTOFs as well as LC-HRMS/MS spectra databases and software that facilitates data interpretation makes the fast and reliable detection, identification and quantification of drugs, drugs candidates, metabolites and degradants, pharmaceutical impurities or new psychoactive substances even in very complex matrices possible. New non-targeted and targeted approaches such as e.g. data dependant fragmentations and all ions acquisitions along with new data processing methods tailored to the analysis needs have been developed and successfully employed for these highly challenging applications.

The examples of applications of LC/QTOF in analysis of drugs will be presented during the lecture. Screening of veterinary drugs in muscles, identification of impurities in pharmaceutical preparations, the detection and identification of drugs and new psychoactive substances, and the study on their collision induced fragmentation will be discussed. The LC/QTOF hardware and software developments will be reviewed.

WEDNESDAY ORALS

PROBING THE LIQUID TO GAS PHASE STRUCTURAL MODIFICATIONS OF IONS

Johann Far, Christopher Kune, Delvaux Cédric, Emeline Hanozin, Denis Morsa, Edwin De Pauw

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The transfer of ions from solution to gas phase is a critical step to produce « native species ». Coming from a highly solvating medium, ionic species will tend to find new equilibrium conformations in the gas phase. The pathway to reach the thermodynamically stable conformation(s) involves crossing potential barriers according to the type of interactions involved. When these barriers are too high compared to the internal energy of the ions, it will result in a “partial memory” (as structural preservation) of the conformation in solution.

In order to evaluate the effect of the solvent evaporation and of the various collision processes encountered by the ions in the mass spectrometer, we developed two strategies:

a) The first strategy consists in comparing in a single experiment the shape of the ions in solution and in the gas phase. A homemade CE-MS low flow interface was built (patent pending) in which CE conditions can be controlled. Data are obtained by coupling capillary electrophoresis with Ion Mobility Mass Spectrometry. Different systems have been investigated, small molecules, complexes, polymers of different topologies.

b) The second strategy consists in probing changes of conformation once the ions are in the gas phase. The ions are rapidly heated by collisions ions during their transfer to the IMS. The heating is obtained by increasing their collision energy, rapidly followed by thermalisation in the IMS cell. The ions may be kinetically trapped in their new conformations. This allows comparing barriers between different ions geometries. The experiments have been performed on nucleic acids (ds DNA), small proteins and sterically blocked peptides (lasso peptides). Disulfide bridged petides of different folds have also been studied. Gas-phase hydrogen-deuterium exchange during the mobility and theoretical calculations were used to validate the structures.

In summary the work intends to evaluate the extent of conformational “memory” of the ions of different nature in view to test the strengths and limitations of “native mass spectrometry”.

RUTHENIUM-ARENE COMPLEXES STUDIED BY ION MOBILITY MASS SPECTROMETRY COMBINED WITH CID TECHNIQUE

Izabella Czerwinska, Johann Far, Denis Morsa, Nicolas Smargiasso, Edwin de Pauw

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In the last few years, monometallic ruthenium-arene complexes have attracted much attention not only because they are versatile and efficient catalyst precursors for olefin metathesis, but they also have being considered as building blocks for new transition-metal-based antitumor agents. The role of the η^6-arene group, as well as the effect of the other ligands on liquid phase chemistry of several complexes have been broadly studied, resulting in a complex activity-structure relationship.

We propose a use of ion mobility mass spectrometry (IMS) and collision induced dissociation (CID) to investigate influence of different ligands on the 3D structure and the reactivity of the ruthenium complexes in the gas phase, giving the correlation with their catalytic or anticancer activity.

The arene ruthenium complexes (tricyclohexylphosphine (1) triphenylphosphine (2) and 1,3,5-triaza-7-phosphaadamantane (3) derivatives) were synthesized as previously described by Laboratory of Organometallic Chemistry and Homogeneous Catalysis (University of Liege). Under experimental conditions, on Synapt G2 (Waters), compound 1 has larger CCS than 2 (Δ CCS = 40 $Å^2$) as the cyclohexane ring has non-planar conformation comparing to hexagonal benzene. Interestingly, under the instrumental parameters used, compound 3 was detected as a broad peak. IMS analysis has revealed two peaks corresponding to two different species with Δ amu = 1.0072. CCS difference between 2 species is quite large, estimated approximately as 30 $Å^2$. To identify the species as well as to monitor the dissociation pathway of both we conducted MSMS experiments. Remarkably, CID studies have shown that the survival yield is significantly different for each species and different fragments were detected in case of $[M]^+$ and $[M+H]^+$. It is worth mentioning that CCS estimated for complex 3 is the smallest, which is attributed to the different PTA ligand size comparing with PPh_3 and PCy_3.

We also investigated series of carboxylic acids derivatives of monometallic-arene complexes by both IMS and CID. The results show that ion mobility mass spectrometry is a good technique to monitor the increase of CCS of the ruthenium complexes according to the increase of the ligands CCS. It was also possible to assess influence of ligand structure on complexes stability by CID MS/MS.

Molecular modelling, including Density Functional Theory (DFT) and Molecular Dynamics (UFF), will be used to generate a range of possible structures, the associated CCS and energies to be compared to the values experimentally derived from IMS and CID.

MAKING THE INVISIBLE VISIBLE: FORMATE ATTACHMENT TO DIVALENT METALLOPORPHYRINS

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Metalloporphyrins and related metallomacrocycles are of utmost importance in a multitude of areas in natural sciences. Moreover, their application in molecular electronics stimulated intense research in recent years across physics, chemistry and material sciences. Progress towards the production of new functional porphyrin-related materials is closely linked to the development of modern mass spectrometry. Both MALDI and ESI have been applied extensively to the analysis of porphyrin-related materials. The two methods are almost complementary, as MALDI analysis does not require solubility and ESI is often softer. In reality, the use of one particular method is often determined by availability rather than performance.

The present study aims at improving the ion formation applying ESI to metallo-porphyrins and –phthalocyanines containing divalent metals. The compounds are a somewhat peculiar case as the two positive charges of the central metal are compensated for by the two negative charges of the very robust and firmly attached macrocycle, without any charged entity releasable to promote ion formation. However, if the oxidation potential is low enough, ESI may lead to oxidation of the porphyrin, resulting in a radical cation. In fact, porphyrins with divalent metals became to some extent the compounds of choice in order to reveal the electrochemical nature of ESI. Nevertheless, oxidation may not occur when the oxidation potential is too high and the aprotic solvents necessary do not support a stable spray at times.

As an alternative analytical approach, we propose the ion formation by addition of the formate anion (HCO_2^-) to the central metal. While focusing on Zn-porphyrins and –phthalocyanines, we show that formate is readily attached to a variety of other metals. A common reaction upon CID is CO_2 loss and transfer of a hydride ion (H^-) to the metal centre rather than electron transfer, as the metalloporphyrin possesses the lower electron affinity. In addition to single porphyrin units, multiporphyrin arrays were successfully analysed. In these, more than one formate anion attaches to the molecule, giving rise to multiply charged species, revealing that one formate anion can bind to two porphyrin units at a time. This latter motif additionally leads to the formation of dimers of the type: $Por_2formate^-$. CID allows to distinguish between isomeric ions, such as Por-HCO_2^--Por vs. Por-Por-HCO_2^-.

GAS-PHASE ASSOCIATION MODES OF MONOPYRENE TWEEZERS THROUGH EXPERIMENTAL AND THEORETICAL APPROCHES

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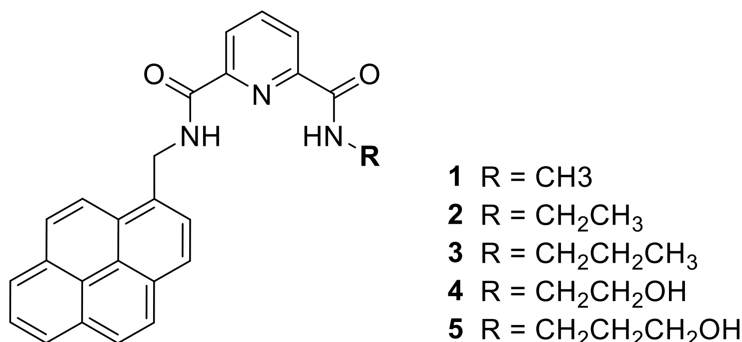
In the solid state and in solution ($CHCl_3$) monopyrene tweezers (Scheme) self-aggregate to form extended supramolecular/chain-like polymeric structure (1) or micelle assembly (2 – 5). The gas-phase association modes of monopyrene tweezers (1 – 5) were the subject of this study.

The modes of aggregation of compounds 1 – 5 were evaluated by the ion mobility mass spectrometry (IM-MS) measurements using quadrupole traveling-wave ion mobility time-of-flight spectrometer (Synapt G2-S HDMS, Waters), molecular dynamic simulations and the PM7 semiempirical calculations.

The results obtained from computational calculations support the results obtained using IM-MS studies. The “micelle”-like conformations are not stable in the gas phase and more extended structures are being formed. Another important conclusion from the MD calculations is that the hexameric “micelle” conformation should be stable in chloroform.



Scheme 1.

IDENTIFICATION OF METABOLITES OF POLYETHER ANTIBIOTIC SALINOMYCIN USING Q-TOF AND HYBRID QQQ-LINEAR ION TRAP

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Salinomycin (SAL) is a polyether antibiotic used widely in veterinary medicine. It is an effective antiprotozoal and antibacterial agent, lately shown to exhibit also antiproliferative activity. Unfortunately, its toxicity to humans is unknown. Different animal species respond variably to salinomycin, which is associated with the routes and extent of its metabolism. Therefore, it seems reasonable to establish the metabolic profile of salinomycin in different species and compare it to the one obtained for humans.

Metabolites of salinomycin were identified in the culture media after *in vitro* exposition of human (HepG2) or rat (FaO) hepatoma cells with the drug. The samples were extracted with acetonitrile in the presence of saturated ammonium acetate solution and analyzed using two different LC-MS systems. The standard HPLC separation was combined with hybrid triple quadruple – linear ion trap detection. The data was acquired using information dependent acquisition (IDA): multiple reaction monitoring signals of theoretical metabolites of salinomycin triggered acquisition of full product ion spectra. The second detection system consisted of microchromatography coupled with Q-TOF mass spectrometry, also operating in IDA mode. The data was analyzed using MetabolitePilot (Q-TOF) and LightSight (QQQ-LIT) softwares.

Fifteen and sixteen potential metabolites were identified using Q-TOF and linear ion trap, respectively but their full characterization was not possible. The detected compounds did not provide specific signals, for each peak a number of SAL-related signals was present. The possible metabolic routes included: oxidation, loss of water, desaturation, internal hydrolysis and demethylation, sometimes in a combination of more than one transformation pathway.

Number of peaks	+ O	- H_2O	- 2 x H_20	-2H	Internal hydrolysis	- CH_3	- CH_3+ O
Q-TOF	6	6	2	4	3	0	0
QQQ-LIT	8	0	0	4	0	5	9

The data obtained with two LC-MS systems is complementary. An unequivocal characterization of salinomycin metabolites is a big challenge due to a complicated structure of parent compound and a low metabolic efficiency of *in vitro* model. Still, the identification of presented potential metabolites enables the quantitative analysis of differences in metabolic profiles between animal species.

The presented research was performed in the frame of the project funded by National Science Centre, Poland (2012/07/D/NZ7/0338).

IMMOBILIZATION OF SILVER ONTO LACTOFERRIN IN LIGHT OF NEW ANTIMICROBIAL APPLICATION

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This work was investigated the physicochemical study on lactoferrin and uptake process of immobilization silver to lactoferrin. A fast and simple methods of lactoferrin analysis are presented in this work. Spectrometric measurements of lactoferrin, its isoelectric point and electrophoretic analysis was applied for protein characterization. Nanocomlex of silver onto lactoferrin was obtained by binding of silver cations to lactoferrin. The infrared spectroscopic study and chromatographic study combined with spectrometric analysis in MALDI TOF/TOF MS system were confirmed metal sorption process. Physicochemical description of immobilization silver to lactoferrin and was carried out such as a crucial point in its comprehension and potential applications in the field of medicine. The application studies were carried out using MALDI MS , flow cytometry and antibiograms tests against selected clinical bacteria. Present study manifested evidence that the silver ions should be effective uptake by native forms LTF from aqueous solution. The kinetic of the silver binding to protein is process heterogeneous process and is carried out in two stages: initial rapid stage with about 70% of the metal bound amount and significant slower second stage with approaching to equilibrium. Obtained results suggest perspectives, in which the nanocomplex of silver with lactotransferrin could be used in the field of medicine and food industry as new alternative and additives to commercial available antibiotics.

Acknowledgement

This work was supported by the National Science Centre (NCN, Poland) Grant No. 2013/08/W/NZ8/00701 (Symfonia-1) and No. UMO-2013/11/N/ST4/01835 (Preludium) as well as from grant “Step in the future” (KWP V)

MULTI-DIMENTIONAL CHROMATOGRAPHIC SYSTEMS COUPLED WITH MASS SPECTROMETRY

P. Stalica

"Shim-Pol A.M. Borzymowski" E. Borzymowska-Reszka, A. Reszka Sp. J.
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MONDAY POSTERS

EVOLUTIONARILY CONSERVED NEUROPROTECTIVE FUNCTION OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) IN DOPAMINE-BASED NEURODEGENERATION

Zrinyi, Z.[1), Petrovics, D.[2), Rivnyak, A.[2), Tamas, A.[2), Mark, L.[2), Kiss, T.[1), Reglodi, D.[2), Pirger, Z.[1), Maasz, G.[1),2)

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a widespread neuropeptide acting as a neurotransmitter, neuromodulator, or neurotrophic factor. It has several well-known physiological function, such as anti-apoptotic, anti-inflammatory, cardioactive and neuroprotective effects. Similar to Drosophila, our earlier findings also showed that a homolog of the vertebrate PACAP38 and its receptors exist in the annelids (Lumbricus terrestris, Lumbricus plyphemus, Eisenia fetida) and snails (Lymnaea stagnalis, Helix pomatia) central and peripheral nervous system. Furthermore, it has important and necessary role in several biological function (feeding, active-inactive state, learning and memory, aging) in invertebrates. Analyses of monoamine were performed with a complex Ultimate 3000 (Dionex) micro HPLC system equipped with a QExactive UHR spectrometer (Thermo Fisher Scientific). Separations were performed on a Kinetex PFP column (100 mm x 2.1 mm, 2.6 µm, Phenomenex). Filters of SIM and MS2 mode were used for selective and sensitive detection of DA and 5HT. The most intense precursor-to-fragment transitions were used for quantitative analysis such as, DA 154.08 → 137.06 m/z, 5HT 177.10→160.08 m/z. Here, using a molluscan and rat model systems, we show the evolutional conserved function of PACAP in dopamine-based neurodegenerative disorder as Parkinson-disease. This disease has been linked to dysfunction of dopaminergic system in substantia nigra. Our aim was to examine the protective effect of PACAP in rotenone (commercially organic pesticide) and 6-OH-dopamine treated invertebrate and vertebrate model animals measuring the dopamine and serotonin levels in their brain. Further aim was to map the proteomic profile in substantia nigra of control, 6-OH-dopamine and 6-OH-dopamine+PACAP injected rats. To the analysis of protein composition SDS- PAGE was carried out, all bands were excised from the gel with a razor blade, digested with trypsin, and analyzed with Waters nanoHPLC (ACQUITY-Waters Corporation) coupled to nanoESI MS (Amazon SL-Bruker) system. Finally, our goal was to observe the changes of metabolic enzymes (catecholamine-orto-methyl-transferase and monoamine-oxidase-B) of dopamine after treatments in both of invertebrate and vertebrate models.

Acknowledgement: This work was supported by PD-109099 OTKA, KTIA_NAP_13-2-2014-0006 National Brain Project and PTE-MTA Lendület Program

ANALYSIS OF THE PROTEIN CROSSLINK-PROFILE CHANGES DURING NEUTROPHIL EXTRACELLULAR TRAP FORMATION

Bernadett Jakob, Endre Kristóf, Krisztián Csomós, László Fésüs and Éva Csősz

University of Debrecen, Faculty of Medicine, Department of Biochemistry and Molecular Biology, Debrecen

Mature neutrophils are the first responders of inflammatory cells migrated to the site of infection in response to the microbial invasion. They are able to attack pathogen directly in three different ways: phagocytosis, release of antimicrobial peptides and neutrophil extracellular traps (NETs) generation. NET is a unique form of cell death in which neutrophils eject their mixture of nucleoplasm and cytoplasm components into the extracellular space forming a web-like structure. Hereby the invaded pathogens are trapped, neutralized therefore their dissemination are inhibited. Several antimicrobial proteins (such as azurocidin, lactoferrin) and proteases (such as neutrophil elastase, myeloperoxidase and cathepsinG) are important constituents of the NET contributing not only to the direct microbial activity but also participating in the proteolytic process.

Many of the proteins participating in NET formation were identified by different research groups. Our aim was to investigate the protein crosslinks and how the protein crosslink-profile changes during NET formation upon different treatments. NET proteins were analyzed by LC-coupled tandem mass spectrometry on a 4000 QTRAP mass spectrometer and based on MS/MS data, the site and the type of crosslinks formed were identified using StavroX protein crosslink examination software. We could demonstrate the changes in the protein crosslink patterns upon various treatments and according to our data it seems that for the crosslinked protein network formation two processes are responsible.

GENERIC LC-MS/MS QUANTIFICATION FOR BIOWAIVER PHARMACOKINETIC TRANSPORT ASSAYS

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Biowaiver pharmacokinetic transporter assays are performed on a wide range of polarity of compounds, chategorized by the Biopharmaceutics Classification System (BCS), to assess a polarity/transport scale as a foundation for comparison. In vitro assay types include PAMPA (parallel artificial membrane permeability assay) and Caco-2 (intestinal epithelial cell based monolayer assay). A set of compounds are used in the in vitro assays to result in permeability data, some of which are challenging in LC-MS/MS bioanalysis. An example test compound is PEG4000 that is a complex mixture of different length of polyethylene glycol chains, complexing alkali and alkali earth metals in different charge states. The distribution of the ion forms of compounds as different m/z species makes the quantification limits insufficient in sensitivity and in selectivity. The solution for the problem is using a generic fragment ion formed in ion source fragmentation. The method was applied for quantitative bioanalysis of PEG4000 with sufficient sensitivity and selectivity for in vitro biowaiver assay samples. Another problematic test compound is mannitol, a high polarity and low ionization compound by ESI. A HILIC method is developed with a generic MS/MS transition, useful to any similar isomer sugar alcohols. Over a dozen other, different polarity compounds were also analyzed with specific LC-MS/MS methods and the results shared hereby.

MASS SPECTROMETRIC DETECTION OF NITRIC OXIDE RELEASE FROM S-NITROSOGLUTATHIONE AND S-NITROSO-N-ACETYL-DL-PENICILLAMINE

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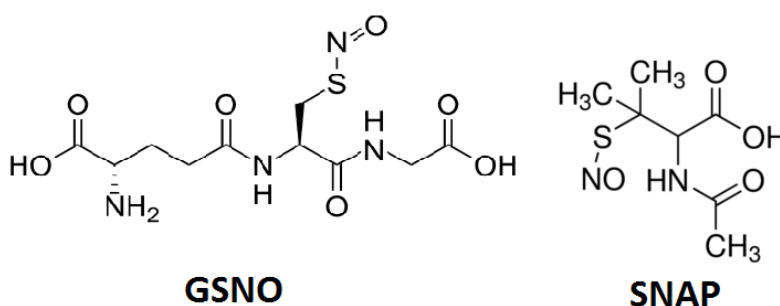
Nitric oxide is a simple molecule of great importance for living organisms. The biological action of NO involves neurotransmission, inhibition of platelet aggregation, vasodilation and cardiovascular signaling. It was proclaimed “Molecule of the Year” in 1992 and the researchers who examined its function were awarded the Nobel Prize in 1998 for discovering its signaling role in blood circulatory system. Recently, NO proved to be an excellent antioxidant in free radical induced lipid peroxidation and an efficient anticancer agent.

Nitric oxide donors are chemical compounds that are able to release nitric oxide (NO) in a controlled manner. The factors which stimulate NO liberation may be light, temperature, acidic or basic environment (pH), catalytic action of enzymes or oxygenation. The NO-release can be also observed in electrospray or MALDI mass spectrometric conditions.

The compounds under research were two known NO-donors, S-nitrosoglutathione (GSNO) and S-nitroso-N-acetyl-DL-penicillamine (SNAP). As GSNO and SNAP play an important role in biological processes, their fate and decomposition are of considerable interest.



The NO release were examined by means of electrospray ionization mass spectrometry in both positive and negative ionization modes and at several values of cone voltage to create a nitric oxide liberation profile.

Financial support from the National Centre for Research and Development under research grant “Nanomaterials and their application to biomedicine”, contract number PBS1/A9/13/2012, is gratefully acknowledged.

HPLC-MS INVESTIGATION OF NANO- AND MICROSTRUCTURES FORMED FROM MONOMERIC AMINO ACIDS PHENYLGLYCINE AND CYSTEINE AS A RESULT OF OSCILLATORY REACTION

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In our previous studies, it has been shown that the low molecular weight chiral organic compounds (e.g., amino acids [1], hydroxy acids [2], and profen drugs [3]) in aqueous or non-aqueous solution can undergo spontaneous oscillatory condensation.

In this study, the achiral high performance liquid chromatography with mass spectroscopy detection (LC-MS), and the light scattering detection (HPLC/ELSD) were used. We also used scanning electron microscopy (SEM), to prove that the pair of amino acids (*L*-phenylglycine and *L*-cysteine) can form nano- and microstructures without addition of any catalyst. The choice of these amino acids was due to their important functions in human body. *L*-Cysteine has a thiol group (-SH), through which it is capable of forming disulfide bond - one of the factors affecting the tertiary structure of the *L*-cysteine derived proteins. This amino acid plays a key role in the body. *L*-Cys combats damaging free radicals, improves the condition of the skin and nails; provides healthy and strong hair, and perfectly nourishes the hair roots and scalp. *L*-Phg is a raw material in the preparation of semisynthetic penicillins, which belong to the group of β-lactam antibiotics.

The obtained results demonstrate that a mixture of *L*-phenylglycine and *L*-cysteine can undergo an oscillatory chiral conversion and condensation, with the consequence that these compounds can form peptide nano- and microstructures in an abiotic system.

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SPONTANEOUS OSCILLATORY REACTION OF PROTEIN AMINO ACIDS IN AN ABIOTIC SYSTEM – THE LC-MS RESULTS

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In previous studies, it has been shown that the chiral low molecular compounds belonging to the group of profen drugs [1], amino acids [2], and hydroxy acids [3] in solution can undergo spontaneous oscillatory reactions. These spontaneous reactions are characteristic of single-component and mixed-component aqueous and non-aqueous solutions.

In presented experiment, we focus our attention on the pair of amino acids (*L*-histidine and *L*-threonine). The goal of this project was to use natural substances (like *L*-amino acids) to spontaneously obtain peptides, without any use of a specialized reaction machinery, extreme temperature, or elevated pressure. The choice of these amino acids was dictated by their important functions in living organisms. *L*-Thr is essential in the synthesis of proteins, contributing to the growth and development of muscles. It also affects the production of elastin and collagen, improving the condition of joints, tendons and skin. Moreover, it supports proper functioning of nervous system. *L*-His acts as a precursor of histamine and it is often present as a key amino acid in active centers of many enzymes.

Firstly, we were monitoring single amino acids (*L*-His and *L*-Thr) by means of HPLC in continuous mode. We also checked the amino acid structures by means of HPLC-MS. With time, there appeared additional structures in both samples, not belonging to amino acid monomers. With that technique, we confirmed the presence of homopeptides in solutions of single amino acids as a result of spontaneous peptidization process. Then, we monitored the amino acid pair (*L*-His–*L*-Thr) by means HPLC in continuous mode and again, we controlled sample by means of HPLC-MS. With that technique, we confirmed the presence of heteropeptides in a binary *L*-His–*L*-Thr mixture as a result of spontaneous peptidization process.

The obtained results showed the oscillatory changes of amino acid concentrations in solutions and were confronted with the predictions of the theoretical model proposed by Epstein et al. [4]. These results are indicative of spontaneous peptidization reactions. An easy access to self-assembled peptide structures is promising for an application to such fields, as biosensing, electronics, medical diagnostics, drug delivery, and tissue repair.

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MS SPECTRA FOR DYES HYDROXYAZOQUINOLINE DERIVATIVES

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The quinolines are one of the most important compounds among *N*-heterocycles and found their board application in pharmaceutical and agrochemical industries [1]. Quinoline was first isolated by F. F. Runge in 1834 during extraction of coal tar [2]. Azo dyes have their application as chemical indicators. They are therefore used in the textile industry as synthetic dyes for staining different textile e.g. cotton, silk, wool or polyester. What is more, this class of compounds found their application in paint industry for the production of paints and varnishes [3]. Chemistry of azo compounds was initiated in 1858 by the German organic scientist Peter Griess, who synthesized 4-aminoazobenzene [4]. Currently, they represent about 60% of all synthetic dyes [5]. Hydroxyazo dyes are popular coloring materials due to their application in various fields of science and technology such as biological and medical research [6, 7]. Hydroxyazoquinolines represent a small percentage of all azo compounds and have been poorly characterizated by spectroscopical analysis.

Our study was carried out to obtain selected quinolines [8, 9]. Six novel hydroxyazoquinoline dyes were synthesized and analyzed by MS and HRMS. The results will be presented on poster presentation.

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SYNTHESIS OF SELECTED 1,10-PHENANTHROLINES BY SKRAUP REACTION; GC-MS INVESTIGATION

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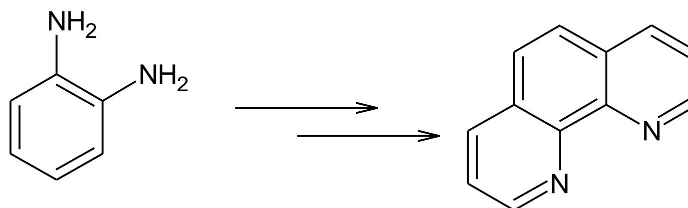
Phenanthrolines are a group of chemical compounds, having two nitrogen atoms in three fused benzene rings (Scheme 1). The most interesting group of phenanthrolines are 1,10-phenanthrolines due to their ability to forming complexes with many metals. They are well soluble materials in many organic solvents.

They are used in analytical chemistry or medicine. In form of iron and cobalt complexes they serve as treatments in anticancer therapy. It is said that the strength of these medicine is even about 100 bigger than popular *cis*platinum [1].

Although 1,10-phenanthrolines are so popular, synthesis of them is complex. K. Panda et al [2] synthesized 1,10-phenanthroline derivatives with good yields. However other researches were unable to repeat their results using Skraup method [3]. In chemistry literature we can find other multi step reaction leading to 1,10-phenanthroline derivatives [4].

Our research is focused on synthesis of 1,10-phenanthroline derivatives within one-step reaction, by Skraup method. We investigated reaction products using GC/MS and NMR methods. The results showed that 1,10-phenanthroline wasn't present among double Skraup reaction products. We proved, that it is highly difficult to obtain 1,10-phenanthroline using Skraup methodology.



Scheme 1. Synthesis of 1,10-phenanthrolines by Skraup method.

On my poster we will show the proposition of reaction mechanism between o-phenylenediamine and crotonaldehyde.

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SYNTHESIS OF SELECTED 1,10-PHENANTROLINES; GC/MS INVESTIGATION

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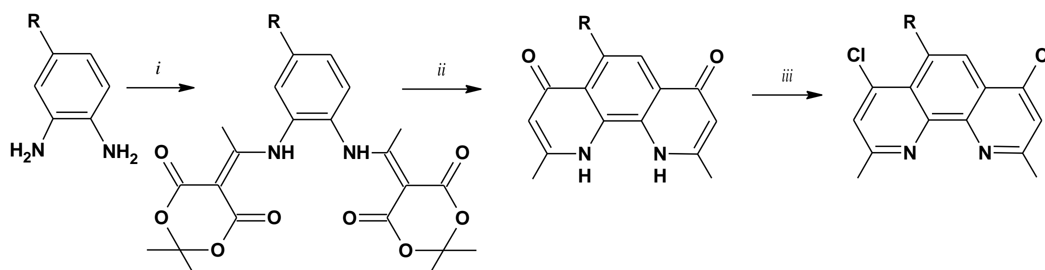
Phenantrolines (o-phenantrolines) is one of the most important class of compounds among N,N-heterocyclic organic compounds. Phenantrolines have nine isomers, differing by the position of the nitrogen atoms, where 1,10-phenantrolines are the most common (Scheme 1). They are composed of two pyridine rings connected with benzene ring. 1,7-phenantroline was firstly obtained by Skraup and Vortmann in 1883 [1]. This class of compounds can be received on various methodologies, including the classical Skraup, Friedlander, Doebner-Miller or Pavarov reactions [2]. Skraup reaction involves aromatic amines and acrolein or glycerin, which required the presence of sulfuric acid and an oxidizing agent as aqueous arsenic acid, nitrobenzene or atmospheric air.

Our research was based on a literature methodology [3]. First step include condensation of Meldrum's acid with trimethyl orthoacetate, and phenylenediamine. Second step involved thermal decarboxylation and cyclization. Finally products were accomplished by deoxygenation and chlorination by refluxing phosphoryl chloride [3].



Scheme 1. Synthesis of selected 1,10-phenantroline derivatives. Reagents and conditions: *i* = Meldrum's acid, trimethyl orthoacetate, reflux; *ii* = diphenylether, reflux; *iii* = phosphoryl chloride.

1,10-phenanthrolines have been widely use as bidentate nitrogen donating ligands in coordination chemistry with numerous applications [4]. They found broad range of application as ligands in transition metal catalyzed reactions, such as iron (II) and copper (I) [5], as luminescent sensors and in photosensitizers for solar cells [3].

The novel 1,10-phenantroline derivatives were synthesized and analyzed by GC and MS. Our recent results will be presented on poster.

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GC-MS IN ANALYSIS OF LAVENDER VOLATILE COMPONENTS

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The essential oils are known for a long time as aromatic vegetable substances which are used to cosmetic, purification and healing purposes. They are widely distributed in the world and are the group of volatile substances of different chemical properties. As far as the general physicochemical characteristics of oils, we can single out: lipophilicity, volatility, optical activity and distinctive smell.

From a chemical point of view, essential oils are multicoponent mixture of monoterpene, sesquiterpene and diterpene compounds and derivative compounds of phenylpropane. Inherence of compounds of hydrocarbons, alcohols, aldehydes, ketones, esters and ethers character in their composition was ascertained. Except above terpene and derivative compounds of phenylpropane, there are also substances of sulfur (gentianaceous oils) and nitrogen, derivatives of acetylene, tropolones, coumarins, organic acids et al. in the oils.

The percentage share of particular components in the oil is variable and it depends on a lot of factors, among other things, plant strain, phase of vegetation, geographic origin of raw material and genetic factors, too. The classification of oils can be made on the grounds of a main component, for example, menthol, thymol, eucalyptol and eugenol. As far as the oil species of plant are concerned, we can find these which contain over 0,01% of oil. The research range incorporated isolating the essential oil from blossoms of lavender (Lavandulae flos), distribution of its components and theirs identification. The isolation of ethereal oil from the plant was made with steam distillation in Deryng's instrument and method of extraction to the 'above the surface' phase (headspace). The components of essential oils were identified on the basis of spectrums of mass which are acquired by technique of gas chromatography connected with mass spectrograph (GC/MS) as well as indexes of retention. The received mass spectrums were compared with data from NIST library.

IDENTIFICATION OF HEXACHLOROCYCLOHEXANE BIODEGRADATION PRODUCTS BY GC-MS

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The majority of organochlorine pesticides (OCP), including hexachlorocyclohexane isomers (HCH), have been banned in many countries due to their mutagenic and carcinogenic properties [1]. However, due to their persistency and lipophilicity, these compounds and their degradation products are still present in the environment, particularly in soil, water, and sediments [2]. Therefore, it is necessary to monitor the soils which are contaminated with OCP, as well as to apply and refine the methods which enable soil remediation. There are many methods used to purify the polluted soils and one group thereof are the bioremediation methods. A vital element favouring these methods is their natural character. As non-invasive methods which do not destroy the structure of soil, they offer a possibility of ground recultivation without its significant and costly transformation.

An important element of the bioremediation processes is identification of the degradation products derived from the organochlorine pesticides. This step allows to check, if the degradation products are not equally or even more environmentally hazardous than biodegradable organochlorine pesticides themselves.

The goal of this study was to chromatographically assess an effectiveness of biodegradation of the HCH isomers by the bacteria strains which have been preliminarily isolated from the grounds polluted by thess pesticides. To this effect, the methods of sample preparation and analysis were devised for the samples polluted with the HCH isomers and with their biodegradation products. For isolation of target compounds, solid-phase extraction (SPE) was used. The analysis of the obtained extracts was carried out by means of capillary gas chromatography with mass spectrometric (MS), as well as electron capture detector (ECD). Usage of the GC/MS technique enabled identification of the products acummulated in soil through biodegradation of organochlorine pesticides. These products were identified on the basis of the acquired mass spectra.

One author (D.Sz.) is the scholarship recipient within the framework of the "DoktoRIS Scholarship Programme for the Innovative Silesia", subsidized by the European Social Fund of the European Union.

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FORMATION OF CURCUMIN MOLECULAR ION UNDER ELECTROSPRAY IONISATION CONDITIONS IN THE PRESENCE OF METAL CATIONS

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Curcumin is a natural compound existing in turmeric, a popular Indian spice, and responsible for its yellow colour. The interest in curcumin results from its biological and pharmacological properties. In particular, the possible application of curcumin in the treatment of neurodegenerative diseases, including Alzheimer's disease, has been recently discussed. It is well known that compounds containing a β-diketone unit form stable complexes with metal cations thanks to the keto–enol tautomerism. Curcumin contains a β-diketone unit and a number of curcumin complexes with metal cation have been studied. Among curcumin–metal complexes, the curcumin–copper complexes have attracted much attention because the copper chelating properties of curcumin have raised hopes of its application as a potent agent for treatment for e.g. Alzheimer's disease.

Electrospray ionisation (ESI) mass spectra obtained for solutions containing curcumin, copper cation Cu^{2+} and other metal cations, namely Co^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+}, have shown an abundant curcumin molecular ion at m/z 368. For the solutions containing curcumin and $CuCl_2$ there is no molecular curcumin ion $[Curc]^{+\bullet}$ at m/z 368. Also, none of the solutions containing curcumin and one of the salts $CoCl_2$, $NiCl_2$, $MnCl_2$, $ZnCl_2$ or curcumin and mixture of these four salts, yielded ion $[Curc]^{+\bullet}$ (a number of ESI mass spectra were obtained at different concentrations of curcumin and metal salt and at different cone voltages). To the best of our knowledge, it is the first example of a system in which copper cations and other metal cations promote formation of organic radical cation under ESI conditions [1].

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COMBINED MODEL MASS SPECTROMETRIC AND QUANTUM CHEMICAL STUDY OF ARTEMISININ-TYPE AGENTS AND ASPIRIN INTERACTIONS WITH MEMBRANE PHOSPHOLIPIDS

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Antimalarial agent artemisinin and its derivatives are currently successfully used to treat severe or multidrug-resistant malaria. They are increasingly being applied also in combination with other drugs, although our knowledge of molecular mechanisms of their action and their main pharmacological properties including their intermolecular interactions with different biomolecules and other drugs is still incomplete. Such information is particularly important in the case of using recommended by the WHO Artemisinin Combination Therapy or application of antimalarial agents together with anti-inflammatory medications. Our previous electrospray (ESI) mass spectrometry (MS) study was related to molecular mechanisms of the artemisinin-type agents antimalarial activity and noncovalent complexes formation between the drugs and heme as their suggested molecular target *in vitro* [1]. In our next study the cationized noncovalent complexes of artemisinin with nucleobases Ade, Cyt and mThy were recorded by ESI MS [2], which model drug-DNA interaction in biological systems that might be related to a recently reported anticancer activity of the artemisinin-type agents.

The current combined model study by ESI MS and quantum chemical calculations is devoted to examining the biologically significant interaction of the artemisinin-type drugs and anti-inflammatory acetylsalicylic acid (aspirin, ASP) with membrane phospholipids molecules. Formation of stable noncovalent complexes of dihydroartemisinin, α-arthemether and arthesunate with dipalmitoylphosphatidylcholine (DPPC) has been revealed by ESI MS probing of the binary model systems (drug:DPPC) with 1:10 molar ratio. Then, triple systems containing the artemisinin-type agent, ASP and DPPC in the molar ratio 1:1:10 have been examined. The results reveal a competition between the antimalarial agents and ASP for binding with the DPPC molecules on the basis of the peaks of the complexes artemisinin-type drugs:DPPC and ASP:DPPC recording in the mass spectra. The complexation between the antimalarial drugs and ASP was also found. The observed phenomenon testifies to the possibility of modulation of the membranotropic activity of arthemisin-type agents and aspirin under their combined usage. The structure and energetic characteristics of the noncovalent complexes registered in the ESI MS experiments were elucidated in the model *ab initio* calculations of dihydroartemisinin and ASP complexes with the polar phosphatidylcholine head of DPPC by DFT/B3LYP/6-31++G** method using Gaussian 09 programme package.

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PROBING MOLECULAR RECOGNITION BY MASS SPECTROMETRY. A CASE STUDY: EXAMINATION OF PROPERTIES OF NONCOVALENT COMPLEXES OF SELECTED MELANOCORTIN RECEPTOR LIGANDS

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The aim of the study is to explore the potential applications of the mass spectrometry to molecular recognition. The overall question addressed in this study concerns the utility of mass spectrometry techniques to provide information on properties of molecules. To respond to this question the features of noncovalent complexes formed between the ligands, that structures are based on the active core of natural agonist of melanocortin receptors and model receptors, are studied using mass spectrometry.

Mass spectrometry-based techniques such as collisionally activated dissociation and ion mobility are employed to study the noncovalent complexes. The simple crown ethers, and more sophisticated compounds are used as model receptors. The ligand structures are based on the minimal sequence required for biological activity of natural melanocortins which is Phe-Arg-Trp.

The preliminary results show that mass spectrometry-based techniques are very valuable tools for the examination of features of noncovalent complexes of the ligands that present different activity to melanocortin receptors with model receptors. The experiments clearly indicate that subtle differences in ligand structures, indistinguishable using mass spectrometry, can be determined with the help of indirect identification, in the form of noncovalent complexes.

MALDI-TOF SPECTRA OF CONDUCTING POLYMERS

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PEDOT (poly(3,4-ethylenedioxythiophene)) and its derivatives are suitable materials as electrocatalysts for detecting organic and biological molecules [1] like dopamine and ascorbic acid [2], pesticides [3], determining cysteine [4]. Electrochemical copolymerization is an efficient approach for the modification of the properties of the PEDOTs, as it yields a variety of conducting polymers with various electrical, optical, and morphological properties [5,6]. Conducting polymers (CP) represent a system which is difficult to analyze with conventional analytical methods because of their insolubility. We applied a method of sample preparation to utilise matrix assisted laser desorption/ionization (MALDI) mass spectrometry. The main goal is to reduce the energy transfer to the analyte molecules during analysis to avoid fragmentations for a reliable analysis of reaction products. The basic structures of the monomers are illustrated in Figure 1.

In the preparation methods, crude powder of insoluble CPs deposits were finely dispersed in solvents, such as acetonitrile (ACN) and ultrasonificated for 2 min. In spite of the lack of solubility of CPs the matrice (1,8,9-trihydroxyanthracene (dithranol)) was directly dissolved in the dispersions of the analytes. Small drops of these dispersed samples were placed on the stainless steel sample stage and the solvent was evaporated prior to measurement.

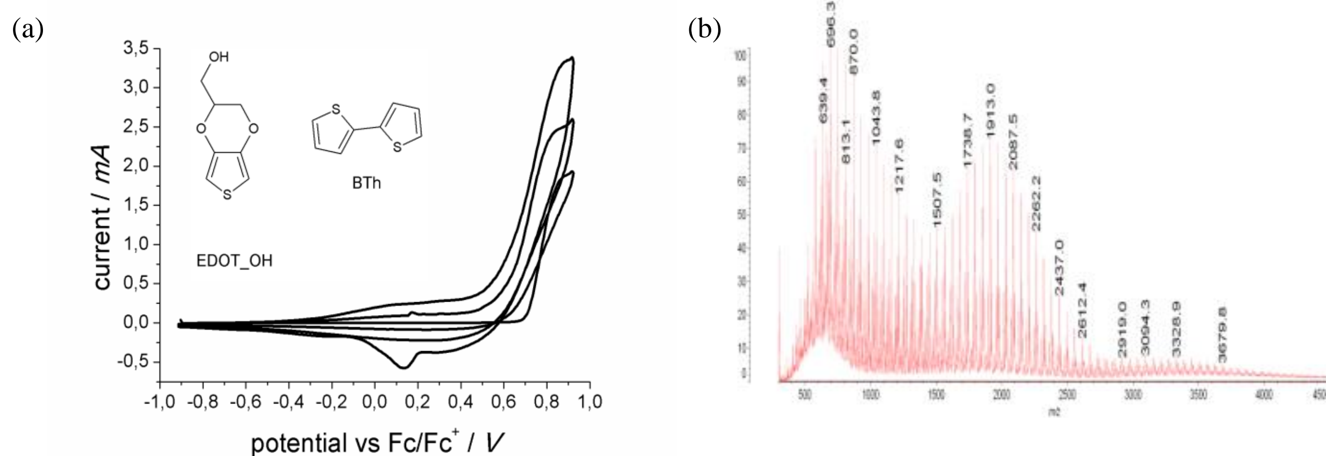

Figure 1. (a) CV of (a) mixture of BTh/EDOT_OH (1:19, mol/mol) in 0,1 M solution of TBABF4 in ACN, (b) MALDI TOF spectra of copolymer p(BTh-co-EDOT_OH).

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GC MS AND ICP MS DETERMINATION OF RESTAURATION FORMULATION USED FOR FIREPLACE CLOCKS

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Background: French fireplaces clocks are antiquitet of very high value.These clocks fireplaces are handmade by old French court artisans from 18th and 19th century and have very sofisticated mechanisams inside.

Objective: Restauration-preserving including tretment of origins of brass and steel surfaces for sheen and scratch patterns, or returning them to this state when excessively altered and keep it from humidity. The aim has to choose composition of unknown formulation used for tretment metal surfaces and to do some improvments in new one.

Methods: ICP-MS, Thermo Scientific iCAP Q with quadrupole analyzer and GC-MS, Thermo Fisher Scientific Trace 1300 GC with ISQ LT MS. For GC-MS method optimisation used: capillary column TG 5MS (15mx0,25mx0,25mm), splitless injection, carrieer gas He 99,999 % with flow 1cm^3/min, oven program T 40-290º C with rate 10 ºC/min from 250-290ºC. Also some other analytical techniques have been used in determination unknown formulation for treatment of these clocks.

Results : ICP MS give concentration of metals in two formulations: Cu 163-179 ppm, Zn 4,46 - 266 ppm, Ag 0,6-3,0 ppb, Hg 1,3-3,3ppb, Na 396-976 ppb, K 130-158 ppb. Sn 2,42-3,02 ppm. Au identified but not quntified because of no CRM solutions of Au. GC MS has been used to split mixture components and to identify it. Analysis of mixture has shown that the most abundant are different type of siloxanes and 2,3-dihidroksi butandionic acid. These compounds come from solutions for coating of steel parts in these clocks.

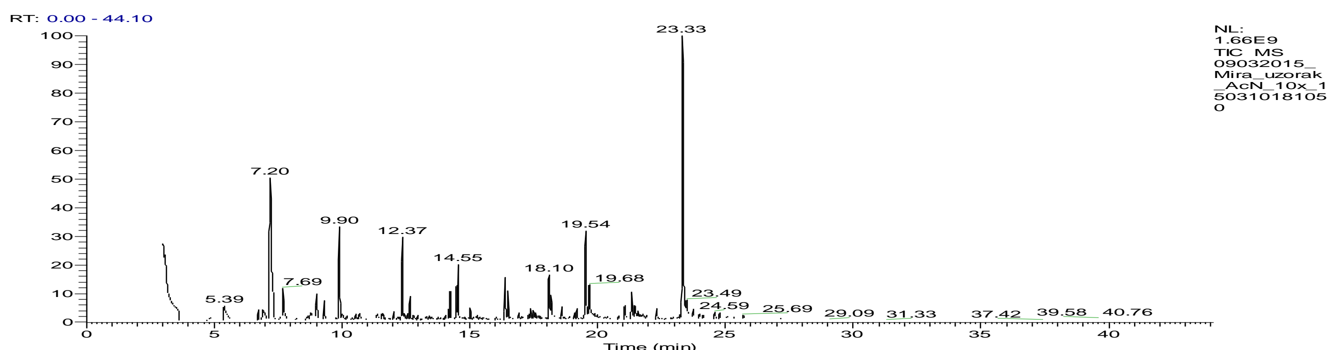

Fig 1 : GC-MS of „old" formulation for steel coating parts in clocks

Conclusion : ICP MS and GC MS with clasical analytical methods all together give an answer that these formulations is based on metal salts , 2,3-dihidroksi butandionic acid , organic solvents and siloxanes compunds.

MOLECULAR ARCHITECTURE OF NOVEL BIODEGRADABLE AND BIOCOMPATIBLE CONTROL DELIVERY SYSTEMS OF SELECTED ANTIOXIDANTS ESTABLISHED BY ELECTROSPRAY IONIZATION MULTISTAGE MASS SPECTROMETRY

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Attaching cosmetics to specific polymer carriers has spurred particular interest. Recently, we elaborated the synthetic strategy for bioactive (co)oligoesters, based on the anionic homo- and copolymerization of β-substituted β-lactones containing bioactive moieties selected from antioxidants used in cosmetics. These novel bioactive polyesters have a larger loading of biologically active substances per polymer macromolecule in comparison to already reported conjugates of oligo(3-hydroxybutyrate) (OHB) with the several selected antioxidants [1-2]. Taking into consideration the prospective use of the synthesized new control delivery systems in cosmetics and their contact with skin, it was important to elucidate the structure and homogeneity of the obtained products.

Herein, we report the application of the electrospray ion trap tandem mass spectrometry (ESI-MS^n) for structural characterization at the molecular level of the novel control delivery systems. The structure of individual macromolecules of the resulting (co)oligoesters (including the chemical structure of their end groups) was determined using the electrospray tandem mass spectrometry technique (ESI-MS^n) supported by ^{1}H NMR spectroscopy. The chemical structure of the individual (co)oligoester chains was also established by investigation of the fragmentation product patterns of the individual molecular ions. The mass spectrometry analysis indicated that the (co)oligoesters obtained via anionic ring opening oligomerization of β-butyrolactone and β-substituted β-lactone with p-anisic moiety contained one up to five bioactive moieties along the (co)oligoester chains.

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GAS-PHASE REACTIONS OF TERT-BUTYL AND CUMENE PEROXIDE ANIONS WITH ALKYL AND ALLYL HALIDES

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Organic hydroperoxides are widely used in organic synthesis, but for now their gas-phase reactions have not been fully examined. In our work we present the reactions of *tert*-butyl and cumene peroxide anions with alkyl and allyl halides. According to literature data reactions between organic peroxides anions and appropriate halides could result either in nucleophilic substitution or elimination where halide anions are leaving groups [1-2].

Continuing the research conducted in our group on the gas-phase reactions of organic anions we studied the reactions of alkyl peroxides anions using mass spectrometry technique and DFT calculations. All experiments were performed on a modified API 3000 (AB Sciex) triple quadrupole mass spectrometer. Alkyl peroxide anions ($R-OO^-$) were generated in solution by deprotonation of an appropriate hydroperoxide in the presence of a strong base (MeONa) and infused to the electrospray ion source. Vapors of halides were delivered to the collision cell of the mass spectrometer where all reactions were carried out.

For all examined reactions only signals corresponding to halide anions were observed. To examine in which way (nucleophilic substitution or elimination) reaction proceeds, DFT calculations were performed. Obtained results show that in the gas-phase elimination reactions are more favored due to lower activation energy.

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CHARACTERIZATION OF INTACT ANTI-HUMAN CYSTATIN C ANTIBODIES, CYST10 AND CYST28, USING THE μLC-MS/MS TRIPLETOF 5600+ SYSTEM

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Human cystatin C (hCC) is a potent cysteine protease inhibitor protein, which participates in amyloid deposits forming. This process play a key role in amyloidoses, a group of disesaes characterized by an abnormal folding of peptides and extracellular proteins [1]. Different monoclonal antibodies (mAb) raised against hCC were shown to prevent its dimer formation with variable strength, thereby indicating potential anti-amyloid aggregation drug candidates [2]. In the presented study two intact anti-hCC monoclonal antibodies: the non-inhibiting hCC dimers formation – Cyst10, and, the most potent inhibitor – Cyst28, were analyzed using the high-resolution Q-TOF mass spectrometer coupled to μLC chromatograph. The μLC-MS/MS method was developed and optimized for intact antibodies analysis on TripleTOF 5600+ system. The comparison of deconvoluted TOF mass spectra enabled to determine molecular weight of the investigated mAbs. The Cyst10 mAb is ~150 kDa (main peak corresponding to mass 150079 Da), whereas the Cyst28 mAb is characterized by lower molecular weight ~146 kDa (main peak corresponding to mass 146513 Da). Obtained results proved also that the antibodies occur in several glycoforms and their glycoprofiles are different. This information provides better insight into the structure of the studied mAbs and will contribute to finding the molecular features crucial for effective prevention of hCC aggregation.

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SIMULTANEOUS DETERMINATION OF TEN ILLEGAL DYES IN ANIMAL FEED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY

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Sudan azo-dyes (like Sudan I-IV, Sudan Red G, Sudan Red 7B, Sudan Orange G, Para-Red, Toluidine Red) are synthetic fat-soluble colorants, used on a large scale in the industry. They are classified as class 3 carcinogens by the International Agency for Research on Cancer (IARC) and are forbidden for use in the food industry. Any amount of these dyes in food can be hazard for human health. Despite this fact, they are still detected in food products, mainly this containing spices like: chili, curry, curcuma or paprika. There were also reports of the use of industrial dyes mixed with feed to enhance the egg yolk color. Due to that, simple and accurate confirmatory methods for the determination of illegal dyes in feed products are needed to ensure animals and consumers health safety. By the authors best knowledge there is only a few methods developed for the determination of Sudan dyes in feed.

This paper presents the development of qualitative and quantitative method for the simultaneous determination of 10 dyes in animal feed by high performance liquid chromatography coupled with tandem mass spectrometry. Separation was achieved in 25 minutes in a gradient elution using acetonitrile (A) and 0.1% formic acid (B). The dyes were extracted with hexane, evaporated to dryness and after the reconstitution of the dry residues analyzed by LC-MS/MS system.

The method was validated [Table 1.] and uses for the homogeneity tests of the in-home prepared feed containing Sudan I at the level of 0.5, 5 and 50 mg/kg. The calibration curves were 0.997 and 0.998 for the working ranges, the repeatability was between CV = 0.38-4.37 %, reproducibility between CV = 0.97-13.92, recoveries ranged from 79 to 99%, LOD was 0.01 mg/kg and LOQ 0.03 mg/kg.

Table 1. Validation parameters of Sudan I

	Validation level [mg/kg]	Working range [mg/kg] / R^2	Repeatability [CV, %]	Reproducibility [CV, %]	LOD [mg/kg]	LOQ [mg/kg]	Recoveries [%]
Sudan I	0.5	0.05 – 1.5 / 0.997	3.52 2.54 3.99	11.42 9.99 9.40	0.01	0.03	88 91 93
	5	0.5 – 15 / 0.998	1.18 0.58 0.38	2.58 0.97 2.55			98 99 98
	50	5 – 150 / 0.998	4.37 1.00 1.88	3.95 9.55 13.92			79 88 92

Validation parameters confirm the methods usefulness for the quantitative analysis of illegal dyes residues in animal feed.

Acknowledgement:
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IMPACT OF DWELL, PAUSE, SCAN EVENT, AND, LOOP TIMES ON MEASUREMENT POSSIBILITIES IN MULTIRESIDUE METHODS

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Antimicrobials for which no Maximum Residue Limit (MRL) was established are not allowed to used in food-producing animals in the European Union [1]. As a consequence it is necessary to carry out official controls of the presence of these banned substances in food of animal origin using methods which are comply with the analytical requirements described in the UE Commission Decision 2002/657/EC [2]. For confirmation purpose a minimum of 4 identification points are required and it can be achieved by use of LC-MS/MS when 1 precursor ion and 2 transition products are monitored in Multiple Reaction Monitoring (MRM) mode.

During development of a method for the determination of 16 sulfonamides and 12 nitroimidazoles in honey by LC-MS/MS significant differences in peak areas were observed when analysing the same sample. To achieve the best possible quality and quantity of data, MS/MS parameters such as dwell, pause, scan event, and loop times need to be optimised. Incorrect settings of these parameters may cause unrepeatable data results or may even lead to under-sampling of LC peak causing the analyte peak is not detected. It is required that at least 20 data points should define each chromatographic peak. Setting optimum dwell, pause, event, and loop times is often a compromise between sensitivity, the number of compounds measured, and speed of analysis.

In this study the intensity and repeatability of peak area measured at different settings of above mentioned parameters of mass analyzer were compared for simultaneous determination of nitroimidazoles and sulfonamides in honey. All chromatographic measurements were performed using a Shimadzu Ultra-Fast Triple Quadrupole LCMS-8050 system. The data collection was performed using event time corresponding to the total time of chromatographic separation of all analytes and using segments mode when selective data collection were applied for events time determined on the basis of the expected retention time of analytes.

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FROM PROTON BONDED DIMERS TO ABSOLUTE PROTON AFFINITY VALUES (PA) OF AROMATIC CARBOXYLIC ACIDS AND APPROPRIATE PHENIDE ANIONS. THEORETICAL STUDY BASED ON DFT AND MULTILEVEL CALCULATIONS.

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Gas-phase acidities (GPA) of organic compounds, and strictly related to them proton affinities (PA) of their anions, are very important thermodynamic parameters for many scientific considerations.

This study contains an extensive dataset of experimentally measured PA values of about 65 aromatic caboxylate anions which were compared with the values calculated using various DFT (B3LYP, PBE0 and M052X) and multilevel (G3MP2, G4MP2) quantum chemistry methods. Furthermore, 62 proton affinities of phenide ions were calculated and the results were compared with PA of the related acids. This part of theoretical study complement lacking values of PA of phenide anions, which cannot be established on the basis of experimental methods.

Moreover, in contrast to experimental methods, quantum calculations of proton affinities of middle size organic molecules are much less time-consuming, yet still very reliable. It was also found that DFT a and multilevel methods give results which are in very good agreement with the experimental ones (RMS% = 0.12 - 0.48).

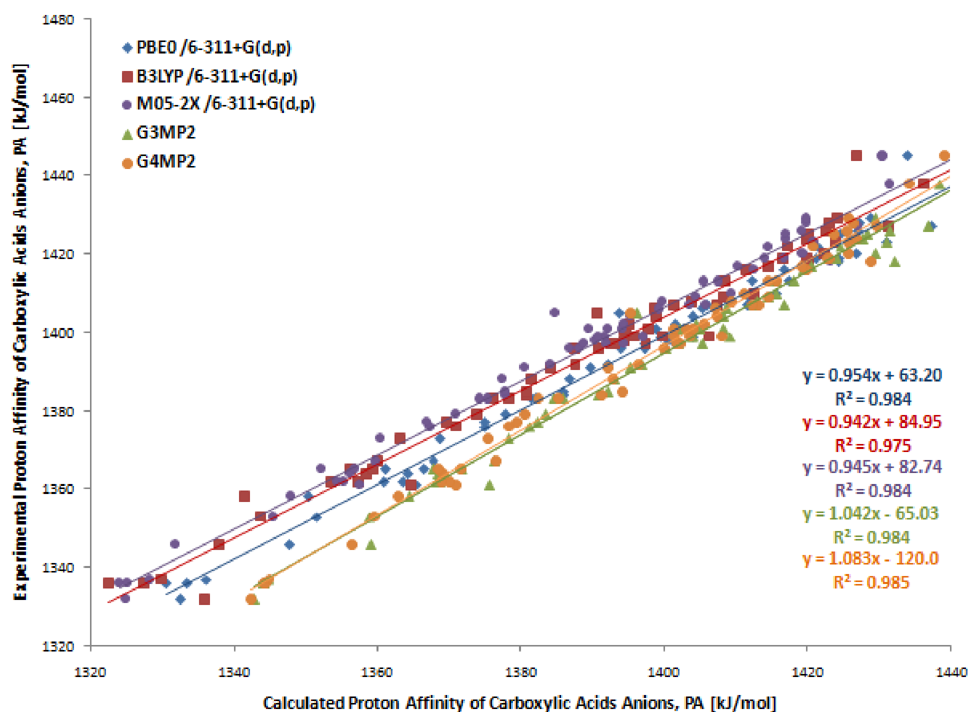

Fig 1 : Correlation between calculated and experimental Proton Affinity values of Carboxylic Acid Anions, in [kJ/mol].

In this work we show a number of PA values obtained using quantum chemical calculations. Quantum chemistry methods appear as an alternative to experimental methods and a source of credible and accurate thermodynamic information.

In our opinion, this statement can be extended to other calculations concerning gas-phase thermodynamic parameters and reaction profiles making quantum chemistry computations an invaluable tool for scientific considerations, especially concerning the gas-phase reactions.

OPTIMISATION OF THE HUMAN BLOOD SERUM FRACTIONATION METHOD FOR THE SWATH-MS ANALYSIS

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Blood serum contains a variety of proteins released from all tissues of the organism. In many cases, changes in the concentrations of particular sets of proteins may be a sign of physiological disorders. Therefore, blood serum constitutes an excellent source of new biomarkers. However, the analysis of such complex samples represents a challenging task. Usually, the most valuable components for biomarker research are present only at nanogram quantities, what makes them concealed by the most abundant serum proteins - albumins and immunoglobulins, which make up more than half of the whole content [1].

Recently introduced to mass spectrometry, the SWATH analysis (Sequential Window Acquisition of All Theoretical Fragment Ion Spectra) allows the label-free identification and quantification of all proteins in the sample [2]. To be successfully performed, this technique needs to be based on an accurate proteomic map, which can be obtained by the IDA (Information Dependent Acquisition) screening of the tested sample kind. However, due to the significant differences in concentrations of distinct proteins, the use of this technique on the serum samples should be preceded by an additional step - fractionation.

In this work, we compared some of the popular fractionation methods (electrophoretic and chromatographic), to establish an optimal protocol for blood serum sample preparation. In the future plans, it will allow us to perform SWATH-MS analysis in search for new biomarkers in the rheumatoid arthritis disease.

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AN OPTIMIZATION OF THE MALDI-TOF ANALYSIS METHOD FOR SERUM LIPIDOME MASS PROFILING IN CANCER RESEARCH

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It is generally accepted that ongoing cancer processes significantly alter the metabolism of cells, and analysis of metabolites allows identifying mechanisms characteristic for cancer. Metabolomics is a field of molecular biology that focuses on global composition and dynamic changes of metabolites and other small molecules. Lipidomics is one of the metabolomic subset that deals with characterization of lipids.

The mass spectrometry allows identification of particular lipids and their quantification in body fluids. For a long time mass spectrometry had not played an important role in lipid research because “traditional” methods of ionization introduced fragmentation of lipid chains. However, the situation changed with invention of the “soft ionization” methods like MALDI and ESI, and MS became a most powerful technique of lipid analysis. Additionally, high throughput of MALDI profiling makes it perfect for large cohort studies, where hundred of samples need to be analyzed. This approach allows identifying many different groups of lipids depending on samples preparation and spectra registration methods.

It is obvious that quality of the measurement depends on the choice of appropriate techniques. There are many different methods that can be used for lipids analysis but none of them is recommended for total serum lipids profiling. It is therefore appropriate to carefully select suitable parameters of lipid analysis by MALDI TOF. In the literature, there are few matrices recommended for lipids analysis with MALDI-TOF mass spectrometry. In the study, we used three different matrices (DHB, DHAP and ATT) in order to find the one, which crystallizes homogeneously, is stable in the vacuum and allows observing many classes of lipids in a positive and a negative ion mode. We also tested the solvents in which the matrix can be dissolved, the order of application of sample and matrix on the target plate.

All the tests were performed for 36 samples. After statistical analysis we decided that for the profiling of cancer serum lipidome, the most suitable are two matrices: ATT (dissolved in 90% ethanol) and DHB (dissolved in 30 % ethanol) in both positive and negative ion modes. Due to obtain homogenous crystallization, matrix should be applied before sample. All of these methods allow to register mass spectra in the automatic and repeatable way.

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NOVEL DERIVATIVES OF CATHINONE CONTAINED IN DESIGNER DRUGS- CHROMATOGRAPHIC AND SPECTROSCOPIC METHODS FOR IDENTIFICATION

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„Designer drugs" are group of substances that affect on the central nervous system and exhibiting psychoactive effects. The measures in question from the point of view chemical are structurally similar to illegal psychotropic substances or drugs for example amphetamine. Lots of them are not included in the Act on the prevention of drug abuse addiction in 2005. They are sold as powders , pills, dried plant names "bath salt", "freshener for toilets", "kindling for the stove", "talisman smile" etc. Among these not prohibited substances can be distinguished group of compounds which are derivatives of cathinone, piperazine, synthetic cannabinoids.

Cathinone (α-aminopropiophenone) is alcaloide contained in *Catha edulis.* Its derivatives, for example pentedrone, 3-methylmethcathinone, ethcathinone, are most commonly found in designer drugs.

On the "designer's drug market" are continually emerging new derivatives which shown psychoactive mechanism of action. We can also observe rapidly emerging cases of poisoning an unknown substances. Selection of appropriate chromatographic and spectroscopic techniques allows for the development of rapid methods to identification psychoactive substances contained in powders or pills.

In this study, we are presented developed method of preparing powdered samples of designer drugs and novel derivatives of cathinone which have been identified by high performance liquid chromatography coupled with mass spectrometer and by mass spectroscopy MS^n with electrospray source of ionization.

FORMATION AND DISSOCIATION BEHAVIOUR OF SALT-LIKE CLUSTER IONS (SODIUM FORMATE) IN ESI-ION TRAP EXPERIMENTS

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Gas phase clusters are often referred to as the fifth state of matter, possessing properties that lie between the atom and the bulk material. ESI is ideally suited to generate a particular cluster type: the salt-like cluster ion of the form cation(cation/anion-pair)$_n^+$. The formation of these clusters takes place in the ESI source and can be regarded as crystallisation occurring during the evaporation of the solvent. If the cluster increases in size, it becomes possible to accommodate more charges on the cluster and ions can be generated of the form: cation$_m$(cation/anion-pair)$_n^{m+}$.

The poster presents a study in which the formation of sodium formate (NaOOCH) clusters were investigated employing an ESI-ion trap from the Bruker Esquire range. Experiments aimed at the manipulation of the cluster formation process, i.e. influencing the size distribution and the respective intensities of a cluster series of a certain charge state. In particular, the ion source parameters are detailed that lead to efficient formation of cluster ions. Of special interest was it to produce the more labile multiply charged clusters.

In a second set of experiments the decay behaviour of the cluster ions was detailed, again the emphasis lay on the di-, tri- and tetracations. It was found that for each series of multiply charged clusters with the same charge state there is a preferred ratio of neutral sodium formate units to charge carrying sodium ions. Depending on the ratio of the cluster being above or below this ratio they fragment either by losing small neutral units or small singly charged ions. The latter reaction is referred to as charge separation or Coulomb explosion. Excited clusters with still sufficient energy may then fragment further until a stable cluster size is reached.

Formation and dissociation mechanisms are discussed in light of the magic number behaviour of the cluster ions, identifying ions and/or neutrals that possess geometries of enhanced stability.

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CLUSTERING OF NITROGEN BASES WITH POLYETHYLENE GLYCOL: ELECTROSPRAY MASS SPECTROMETRY AND COMPUTER EXPERIMENT

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Study of interactions and clustering of biomolecules with organic polyether polyethylene glycol (PEG) is of interest for elucidation of molecular mechanisms of self-assembly of pegylated nanoparticles designed for drug delivery. While electrospray ionization (ESI) mass spectrometry is a recognized tool for detection and identification of noncovalent complexes of biomolecules, the correlation between associates present in the system under study and clusters recorded in the mass spectra is still under discussion [1].

In the present work we have applied ESI and molecular dynamics (MD) computer experiments to prove formation of stable noncovalent complexes of protonated nucleic acid bases cytosine (Cyt) and adenine (Ade) with oligomers of PEG-400. Interaction of these compounds by formation of sets of abundant clusters $PEG_n•Cyt•H^+$ and $PEG_n•Ade•H^+$ (where the degree of polymerization n = 3 - 17) was observed under ESI conditions.

MD computer experiments have demonstrated how a self-assembly of the clusters from the individual components proceeds in the gas phase by winding of a polymeric chain around the organic cation, acquiring quasi-cyclic or quasi-helical conformation. To prove the existence of such clusters in the liquid phase the dynamics of the performed $PEG_n•Cyt•H^+$ associates in methanol or water droplets was monitored; preservation of the complexes with minor changes of the polymeric chain conformation due to thermal motion was observed. To prove the survival of such clusters on their transition from the liquid to the gas phase under ESI conditions, the disintegration of methanol droplets was simulated by MD. To mimic this process, nanodroplets composed of 3000 methanol molecules and the preformed $PEG_n•Cyt•H^+$ complex (n=4, 8) were allowed to expand into a low pressure (10^{-2} Pa) medium. Evaporation of separate methanol molecules was observed first followed by the expansion of the droplet and its fission in the form of methanol clusters where $PEG_n•Cyt•H^+$ associates remained solvated for a noticeable time. The base-oligomer cluster did not decompose during the completion of methanol solvent evaporation; the steps leading to release of stable gas-phase conformation followed the desolvation of the complex.

Thus, MD simulation has proved that the gas-phase clusters recorded in the ESI mass spectra reflect adequately noncovalent oligomer-base complexes present in the initial solution.

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MATERNAL-EMBRYO LIPID NETWORKING DURING THE EARLY STAGE EMBRYOGENESIS

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The receptive phase of uterus is marked by structural and functional maturation of endometrium. This is a limited time span when the blastocyst competency is superimposed on the receptive endometrium. The lipid metabolism and signaling of early stage pregnancy are of a vital importance in successful embryogenesis. However, the embryo-maternal molecular networking is not well understood.

Matrix-assisted laser desorption ionization (MALDI) imaging mass spectrometry (IMS) is a new developed technique that enables the evaluation of molecular signals direct *in situ* from the tissue surface or thin sections. MALDI IMS is a label-free technique with the ability to visualize the distribution of even hundreds of biomolecules in a single measurement, maintaining the morphological integrity of the intact tissue by avoiding homogenization. Although previous IMS studies have provided outstanding spatiotemporal profiles of phospholipid and protein changes during the embryo implantation, the discovery of the *in situ* local molecular alterations during the early stage embryogenesis needs further investigations.

In our study we investigate distributions of lipids in different developmental stage embryos, to recognize the alterations of molecules during implantation and early embryogenesis. In our examination we explored significant differences in the composition of lipids which are regulated by LPA2 and COX2. The greatest differences were discovered the local distribution of phospholipids where the spatial and temporal alterations of phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol seem to follow the enzymatic alterations of embryo and endometrium. Our results contribute to understanding of early embryonic development and the method we developed provides new possibilities for further research.

NEW SALIVARY BIOMARKERS OF SCHIZOPHRENIA

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Schizophrenia is a severe and debilitating neuropsychiatric disorder with an increasing lifetime risk. Elucidate the unknown etiology could help to mitigate both personal and social loss of values.

Cerebrospinal fluid (CSF) and blood as human body fluids have become generally used in proteomic studies of Schizophrenia (SCZ). Human saliva also contains informative components, namely proteins that can be useful as diagnostic biomarkers.

In this study mass spectrometry–based proteomics (MALDI TOF/TOF MS, LC-MS/MS) combined with 1D/2D gel electrophoresis workflow were used for identification of differentially expressed salivary proteins. Eighteen schizophrenic patients (paranoid type) were matched to control group (n=18). The identified biomarkers are functionally classified into energy metabolism, oxidative stress, cytoskeletal, synaptic, signaling and inflammation groups.

These results may support previous findings about the development of psychosis and demonstrates that whole saliva includes several potential molecular markers for aiding the diagnosis and treatment of SCZ. Nevertheless, in contrast to CSF and blood sampling, saliva collection is a non-invasive, simple, safe, stress free and painless technique.

LOCAL EXPRESSION OF PROTEINS IN THE TUMOR MICROENVIRONMENT OF HEAD NECK SQUAMOUS CELL CARCINOMA

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Head and neck squamous cell carcinoma includes a diverse group of tumors from the upper aerodigestive tract with high morbidity and mortality. Additionally, the heterogeneous microenvironment of the cancer cells, characterized by chronic inflammation and consisting of various components including neostroma, growing blood vessels and infiltrating immune cells, is of a vital importance in cancer development and behavior. Because of this cellular and molecular diversity no reliable clinical markers were validated to diagnose the early stage HNSCC.

In this study, we used MALDI mass spectrometry for molecular profiling of presented proteins in tissue samples and human saliva. The imaging mass spectrometry results were validated by immunohistochemistry and SDS-PAGE based MALDI TOF/TOF MS proteomic investigations.

Our results showed that S100A8/A9 proteins are presented in a great extent along different histones in the tissue samples as well as human saliva. The results suggest that these proteins could be potential biomarkers. Moreover, the overexpression of S100A8/A9 proteins is localized in the cancer cells and in the supportive stromal regions but there is no attendance in the healthy tissue areas.

The upregulation of calgranulins in the neoplastic and hyperplastic epithelium as well as their salivary secretion suggest that the S100A8 and S100A9 proteins are potential biomarkers for very early stage tumorigenesis in HNSCC.

RELATIVE QUANTIFICATION OF THE HUMAN NOD-LIKE RECEPTOR FAMILY CARD DOMAIN CONTAINING 5 (NLRC5) PROTEIN BY TARGETED MASS SPECTROMETRY APPROACH

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Pattern recognition receptors of the innate immune system have key roles in the recognition of pathogen- and danger-associated molecular patterns. The NLRC5 protein is the largest member of the Nod-like receptor family which typically acts in the intracellular space. NLRC5 regulates the inflammatory pathways via the regulation of NF-κB protein forming a negative feedback of the inflammatory processes. NLRC5 have role in the inflammasome signaling and in the regulation of MHC I expression as well. Several studies have characterized the NLRC5 by gene expression analyzes and to further study the NLRC5 protein our workgroup has tried to establish an antibody-based approach using several anti-NLRC5 antibodies, but the tested antibodies were found to be unspecific. To overcome the antibody specificity problem we have developed a so called mass spectrometry Western blot technique, a Selected Reaction Monitoring (SRM)-based targeted mass spectrometry approach for the analysis of the NLRC5 protein. This technique provides an alternative antibody-free method for the analysis of proteins. For the identification of potential peptide targets NLRC5-transfected HEK 293T cells were analyzed by LC-MS/MS. Peptides corresponding to the NLRC5 protein were further analyzed by BLASTp in order to ensure the uniqueness and specificity of the identified peptides. As a result of the LC-MS/MS and BLASTp analyzes we have identified three NLRC5 specific peptides. SRM assay was designed and stable isotope-labeled synthetic (SIL) peptides were administrated. The developed SRM experiment was tested on HEK293T cells as negative controls and NLRC5-transfected HEK 293T cells as positive controls. While the SIL peptides were detected in all experiments, the endogenous peptides were not present in the negative control samples indicating the specificity of the developed SRM experiment. As far as only one endogenous peptide was detected in the positive control samples, a relative quantification of the NLRC5 protein was performed in HaCat, HeLa and THP-1 cell lines. The endogenous/SIL ratio showed significant differences between the analyzed cell lines, THP-1 cells expressed the highest amount of NLRC5, while HaCat cells contained the least amount of this protein. Based on our results, we have successfully developed a targeted mass spectrometry-based approach for the relative quantification of NLRC5 in complex biological samples.

MASS SPECTROMETRIC SEARCH FOR SILVER NANOCLUSTERS IN CARBON NANOTUBES-SILVER COMPOSITE

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Combination of two nanomaterials – carbon nanotubes (CNT) and silver nanoparticles (AgNPs) – into a new nanocomposite is expected to combine useful properties of both materials promising for nanotechnology, pharmacological and biosensor applications. Existing methods of decoration of CNT surface by silver involve utilization of reducing agents applied in standard methods of AgNPs production. Recently a method of CNT-AgNPs nanocomposite fabrication by ultrasonication of water suspension of such reagents was proposed [1]. In our previous works [2, 3] we have demonstrated that silver nanoclusters Ag_n can be formed under certain conditions without participation of the reducing agents. Having this in mind, we have proposed and tested a method of CNT-AgNPs production by mere ultrasound treatment of aqueous suspension of CNT with $AgNO_3$ salt without any additives in the assumption that reactive species formed in the cavitation process are sufficient for initiation of silver ion reduction necessary for AgNPs formation. A mixture of 0.1 mg/ml CNT and 20 mg/ml AgNO3 in distilled water was sonicated at 22 kHz for 30 minutes; as a result, stable dark suspension was formed. Presence of dense particles within the CNT mesh was confirmed by electron microscopy.

We have applied Laser Desoption/Ionization (LDI) mass spectrometry to prove formation of silver aggregates. It was shown earlier that Ag monomer or dimer can be sputtered by LDI from the pure $AgNO_3$ salt; mainly abundant Ag^+ ion is usually sputtered from the bulk metal silver surface. Nanoparticles of silver can be the source of silver nanoclusters Ag_n^+ (where n is up to several tens of atoms) production. Although larger AgNPs are too heavy to be volatilized by the LDI, a possibility of sputtering of abundant Ag_n^+ clusters can be considered as a criterion of AgNPs presence in the sample [2]. We have observed that the LDI mass spectrum obtained from the CNT-Ag material produced by sonication contained a set of Ag_n^+ nanoclusters (n=1-9) with “magic” (more intense) odd n numbers. Thus, formation of CNT-AgNPs nanocomposite was proved.

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CELL ENERGETICS WITH HPLC-MS

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The energy within cells can be stored and transported in the form of several molecules. Different physiological and pathological states of the cells mean different distribution of the energy.

The first and easiest way to get information about the energetic state of a cell culture is measuring the concentration of its nucleotides. Nucleotides are also involved in cell signaling, in cancer and in many other disorders, so it is essential to have reliable and sensitive methods to measure them. A further important molecule in cell energetics is alanine. Alanine plays an irreplaceable role in the Cahill cycle. In this work a high performance liquid chromatography-mass spectrometry experiment is presented where I use ionpairing chromatography to show the hyperglycemic rearrangement of the energetics of a mouse myoblast cell line. The main disadvantage of using ionpairing coupled with mass spectrometry and the applicability with an other cell line is also shown in the poster.

MOLECULAR DIVERSITY AND BODY DISTRIBUTION OF SAPONINS IN THE SEA STAR *ASTERIAS RUBENS* BY MASS SPECTROMETRY

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Introduction :

Saponins are natural molecules that the common sea star Asterias rubens produces in the form of steroid glycosides bearing a sulfate group attached on the aglycone part. These molecules reveal a large chemical diversity and different biological activities. The general objective of this project is to link specific saponins with selected biological activities of the starfish Asterias rubens. In this context, we determined the saponin inter-organ variability by mass spectrometry, 5 organs have been selected for the present work.

Methods :

Amongst all the available analytical methodologies, mass spectrometry is definitely a first-choice technique for tackling the large diversity of saponins in echinoderm tissues. MALDI-ToF experiments were selected as the primary tool for a rapid screening of the saponin mixtures, whereas LC-MS(MS) techniques were used to achieve chromatographic separation of isomers. Spatial distribution of saponins within the organs of A. rubens has then be probed by MALDI Imaging analysis (University of Lille, Laboratoire de Spectrométrie de Masse Biologique, Fondamentale & Appliquée).

Results :

First of all, on the basis of all the collected MSMS data, our analyses demonstrated that the diversity of saponins is higher than previously reported [1]. Secondly, the comparison of the saponin contents from the five body components revealed that each organ is characterized by a specific mixture of saponins and that between animals there are also qualitative and quantitative variability of the saponin contents which could be linked to the sex or to the collecting season [2]. Thirdly, the MALDI Imaging analyses allow to localize saponins on a cross-section of sea star arm with a good resolution. Particularly, these molecules are preferentially localized in the surface layers (i.e., mucus layer) of the body wall and tube feet. This distribution, together with the lack of saponins in the fooprints, suggests that saponins would be involved in defense rather than in locomotion in the sea star Asterias rubens.

Conclusions :

The observed high variability of the saponin contents, in terms of organs and animals, unambiguously confirms that saponins probably fulfill several biological functions in A. rubens. The results of the present report will pave the way for our future studies that will be devoted to the clarification of the biological roles of saponins in A. rubens at a molecular level.

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A TANDEM MASS SPECTROMETRY-BASED METHOD TO ASSESS THE ARCHITECTURAL PURITY OF SYNTHETIC POLYMERS: A CASE OF A CYCLIC POLYLACTIDE OBTAINED BY CLICK CHEMISTRY

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Click reactions have recently attracted increased attention, specifically for use in materials and life sciences. In particular, the design of cyclic peptides, polynucleotides and more generally cyclic polymers has been drastically improved using click reactions. As polymer physical properties are inherently related to the chemical structure, the architectural purity assessment is a key step in their evaluation. The traditional characterization techniques, such as proton nuclear magnetic resonance (1H NMR), size-exclusion chromatography (SEC), and Fourier Transformed Infra-Red (FT-IR) spectroscopy, are generally not sensitive enough to characterize unambiguously the purity of cyclic polymers and provide only averaged information over the sample. Usually, mass spectrometry (MS) measurements can provide far more detailed information on the chemical structure of individual polymer chains as well as side-products with a high sensitivity. Nevertheless, as a consequence of the 100% atom economy of click reactions, linear and cyclic polymers are isomers and therefore the purity cannot be unequivocally assessed based on single-stage MS measurements. The development of high-sensitive and discriminative characterization techniques is therefore of interest. In this context, a tandem mass spectrometry-based method is developed to determine the degree of purity achieved in the cyclization of a linear poly(L-lactide) prepared by copper-catalyzed alkyne-azide cycloaddition (CuAAc). When proton nuclear magnetic resonance, size-exclusion chromatography, and single-stage mass spectrometry are unable to demonstrate the presence of a residual linear polymer, the proposed ESI-tandem mass spectrometry methodology allows detecting starting material traces (<5%) based on radically different collision-induced dissociation (CID) behaviours. Thanks to its ultrahigh sensitivity, mass spectrometry is emerging as a predilection technique for the analysis and quantification of numerous isomeric pairs of macromolecules, particularly at the trace level.

ANALYSIS OF β-LACTAMS BY ION MOBILITY – MASS SPECTROMETRY AND THEORETICAL CALCULATIONS

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The β-lactams are among the best known and extensively studied heterocyclic ring systems due to their biological activity. [1] They are part of a large group of compounds exhibiting bactericidal activity, so-called β-lactam antibiotics. These include penicillins [2], cephalosporins [3], carbapenems [4], and monobactams. [5]

The aim of the study is to identify and separate between six diastereoisomeric β-lactams using ion mobility technique coupled to mass spectrometry (IM-MS). Additionally, the theoretical calculations were performed to support the results obtained by IM-MS.

IM-MS measurements were conducted on a quadrupole ion mobility time-of-flight instrument (Synapt G2-S HDMS, Waters). T-Wave ion mobility was calibrated using polyalanine and drug-like compounds. Optimized structures of β-lactams were generated using molecular dynamics (MD) combined with DFT method (M052x/6-31g(d,p) level of theory). Theoretical collision cross-sections of β-lactams were calculated using the open source software program MOBCAL. [6]

We demonstrate the first positive results of the IM-MS separation of sodiated $[M+Na]^+$ diastereoisomeric β-lactams. The MD-DFT theoretical calculations yield the optimized structures of the analyzed compounds. Theoretical collision cross-sections are calculated for the various model structures and are compared with the experimentally derived values.

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TUESDAY POSTERS

MASS SPECTROMETRY-BASED RELATIVE QUANTITATION OF CHRONIC KIDNEY DISEASE PLASMA PROTEINS USING ITRAQ AND LABEL-FREE STRATEGIES

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Quantitative proteomics research aims to identify and quantify the dynamics of protein abundance and is one of the most effective methods for analyzing changes in proteomes of diseased cells and tissues. In shotgun proteomic methods involving iTRAQ (isobaric tags for relative and absolute quantitation) labeling, peptides from different biological samples are first covalently bound to a set of different chemical "tags" with identical masses (i.e., isobaric tags) so that a mixture of labeled peptides can then be identified through MS/MS. The relative peptide accumulation can be quantified by measuring the relative abundances of the individual isobaric tags. Label-free quantitative proteomics approaches are based on changes in peak areas of peptides derived from the LC separation. Proteins are identified after MS/MS analysis. Here, we systematically used both approaches and implemented a comparative proteomic analysis of blood plasma from patients in various stages of chronic kidney disease (CKD) (n=120), patients with advanced cardiovascular disease (CVD) (n=40) and healthy volunteers (n=30). Plasma proteins were prepared for MS analysis and obtained mixtures of peptides were analyzed using Q Exactive Hybrid Quadrupole-Orbitrap coupled to Dionex Ultimate 3000 NanoLC (Thermo Scientific).

The accuracy, reproducibility, number of identified/quantified proteins and statistically significant protein differences, as well as efficiency of these two quantitative proteomics a were evaluated and compared. Differential proteins detected using iTRAQ and label-free approaches were precisely analyzed. Obtained results showed that both iTRAQ labeling and label-free strategies provide high quality qualitative data. 947 and 969 proteins were identified using label-free approach and iTRAQ approaches, respectively.

However, heterogeneity in quantitative results was observed between both approaches. On the basis of obtained results we conclude that both methods have some advantages and disadvantages. Finally, we provide some guidance for selection of the appropriate method for a quantitative proteomics study.

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MOLECULAR CHARACTERIZATION OF ORAL SQUAMOUS CELL CANCER AND ADJACENT TISSUES BY MALDI-IMS

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Objective: Surgery is the primary treatment in oral squamous cell carcinoma (OSCC), one of the most frequent cancers in head and neck region. However, there is a significant risk of local recurrence in some OSCC patients with histologically negative surgical margins. Hence, determination of tumor-specific molecular factors in resected areas appears a vivid issue in this type of cancer, where excessive margins cannot be practiced. Here we aimed to characterize molecular profile of tumor and adjacent tissues in clinical material from patients with OSCC.

Methods: Tissue samples (tumor and adjacent areas) resected from 8 patients with OSCC were fresh-frozen, cut in cryostat, placed on ITO-coated glass slides, and then dried in vacuum and covered by the methanol solution of 2,5-dihydroxybenzoic acid (50% methanol, 30 mg/mL DHB, 0.2% TFA) using ImagePrep device. Samples for peptide analysis were incubated over-night with trypsin prior to matrix deposition. Spectra were registered using UltrafleXtreme MALDI-ToF spectrometer in positive reflectron mode with 100 µm raster; peptide and lipid spectra were recorded in the 800-4000 Da and 300-1500 Da range, respectively. Tryptic peptides were identified in tissue lysates using LC-MS/MS approach and annotated to IMS data.

Results: Supervised analysis of tissue areas pre-defined by pathologist resulted in identification of molecular components that differentiated OSCC from adjacent tissues. Several peptide and lipid ions, which levels were significantly different in cancerous and normal epithelium were detected. These were exemplified by peptides (m/z values) 930.55, 2105.02, 2336.16 Da up-regulated and 818.56, 863.53, 937.71 Da down-regulated in cancerous epithelium in comparison to normal epithelium. Further unsupervised analysis resulted in identification of proteome and lipidome components associated with different regions of tumor.

Conclusions: MALDI-IMS allows identification of molecular components, both protens and lipids, that differentiate cancerous and normal epithelium, as well as different areas of oral tumor tissue.

TROUBLESHOOTING SAMPLE PREPARATION OF *DENDROLIMUS PINI* SEX PHEROMONE FOR MS ANALYSIS

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Dendrolimus pini is a defoliating pest of pine forests in Europe, Georgia, China and a few North-West African locations [1]. The moth has one generation per year under normal conditions. The real pest are caterpillars which grow from a tiny 0.5 cm after hatching up to 5-8 cm when mature. A mature specimen can chew a pine needle of its length just in a minute. Adult insects do not feed – their single role is to mate and lay eggs that begin the next generation of *D.p.* To attract males, the female adults emit sex pheromone they produce within specialized tissue and do not store it but immediately secrete to the outer body surface for evaporation. The known components of the pheromone are (Z,E)-5,7-dodecadienal and (Z,E)-5,7-dodecadien-1-ol secreted at the estimated rates of few nanograms per hour. The lure commonly used by forest sevices to control *D. pini* consists of aldehyde and alcohol in the 6:4 mass proportion. The composition has not proved sufficiently effective, so the search was set for missing components of the pheromone, based on the mass spectrometric analysis. Sampling of the pheromone for the analysis appeared a challenge that is presented on this poster.

Female pupae of *D.p.* stripped off cocoons were obtained from the Forest Research Institute in Sękocin, Poland. Each pupa was placed in a separate glass container furnished with a support mesh made of acid resistant steel and a perforated lid made from alu foil. The containers were kept in ventillated terraria equipped with time controlled LED lighting providing 17-hours days and 7-hours nights, with 1-hour dusk and dawn periods. The samples were collected fom calling females using either the flow-through or stationary sampling vessels equipped with various samplers (charcoal beds, dry ice-acetone cooling traps, 2,4-DNPH beds, various SPME cartidges: PDMS, PDMS-DVB, PEG, CAR-PDMS and PA). In addition, abdominal parts of the females were excised from living specimens for the headspace and solvent extraction analyses (before the preparation, the females were cooled down for a while at -20 ^{o}C). The poster compares the effectiveness of sampling systems and particular samplers used, as well as the influence of the insect condition on the quality of the samples collected. The contradictions with available literature are explained.

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ANALYSIS OF INSECT SIGNALLING USING ION-TRAP MASS SPECTROMETRY A CASE OF *DENDROLIMUS PINI*

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Understanding of insect signalling provides effective tools for pest control. For instance, artificial sexual attractants can be used to lure male insects to traps rather than let them find the calling females. Ion-trap mass spectrometry coupled with a capillary gas chromatography is an efficient tool for analysing blends of volatile substances such as sex pheromones. This work shows the analysis of sex pheromone of pine-tree lappet moth (*Dendrolimus pini*), a serious pest of pine forests in Europe and Asia. This species belongs to a large family of Lepidoptera that comprise the second largest insect pest of coniferous trees.

(a) (b)

Fig. 1. (a) Male and (b) female of pine-tree lappet moth (*D. pini*).

Caterpillars of pine-tree lappet moth are the most dangerous defoliators of pine trees in Poland. On average, each caterpillar consumes 900-1000 needles, which destroys the assimilation apparatus and weakens the trees making them vulnerable to secondary pests. The straightforward consequence of this damage is the death of pine forests. One of the efficient and environment-friendly methods for the forest protection against *Dendrolimus pini* pest takes advantage of disturbing the mating flight with synthetic sex pheromone lures. The pheromone lures available so far are based on substances discovered in the early 1980s. They were tested in many countries and appeared rather inefficient in the forest protection.

Thus, a project was started to discover the full composition of the sexual pheromone of pine lappet moth (*Dendrolimus pini*) and to provide an improved analogue of this pheromone for better forest protection. After a challenging search for an effective method of sample collection (see a companion poster by Rudziński et al.), a variety of SPME samplers was used in a stationary sampling system. Namely, the following SMPE fibres were evaluated: polidimethylsiloxane (PDMS), carboxen-polidimethylsiloxane (CAR/PDMS), divinylbenzene-polidimethylsiloxane (DVB/PDMS), polyethylene glycol (PEG) and polyacrylate (PA). In the first phase of the study, the fibres were evaluated in a static headspace mode against the authentic standards of 5E, 7Z-C12-OH and 5E, 7Z-C12-CHO that are the recognized components of the sex pheromone of *D. pini*. Then, the pheromone samples were collected from living calling females of *D. pini*, with SPME fibers placed not more than 4 mm from the extruded ovipositor of each insect. The GC/MS-IT analyses of adsorbed analytes were carried out using a Thermo 1300 GC gas chromatograph coupled with ITQ 700 ion-trap mass spectrometer with 70eV EI ion source. Our results show which of the SPME cartridges used are appropriate for identifying the individual components of sex pheromone blend that the female pine-tree lappet moth emits.

EXPLORATION OF AQUEOUS ORGANOSULFATES FROM ISOPRENE BY LC/MS/MS

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Nowadays, the airborne particulate matter gives rise to the most serious air pollution worldwide. Not only does it affect the Earth's climate but above all deteriorates the health and well-being of the mankind. According to the WHO data, there is a clear correlation between the long time exposure to air containing increased concentration of fine and ultrafine aerosol particles and numerous cases of asthma and cardiovascular diseases.

The mechanisms underlying the formation and growth of aerosol in ambient air has been a subject of intense research during the last decade. However, there is a gap between the results obtained in the laboratory and field frameworks. Our present knowledge of the aerosol formation is highly limited owing to the complexity of the aerosol chemistry and the constraints of the analytical methodology.

The continental atmospheric fine aerosol matter is by far dominated by organic compounds. Up to date, different classes of organic compounds, including alcohols, carboxylic acids and esters, have been determined using hyphenated mass spectrometry. However, little is known about the complete composition of the polar aerosol fraction which plays an important role in the particle formation and growth.

In this poster we would like to present our recent findings on the molecular characterization of novel aerosol components formed in the aqueous-phase processing of isoprene initiated by sulfate radical anions. Isoprene (2-methyl-1,3-butadiene) is one of the most important non-methane hydrocarbons produced by plants and emitted to the atmosphere at a high rate (500-750 TgC/year). Once released, it interacts with other reactive species present in the atmosphere, such as O_3, $HO^{\cdot}$ and $SO_4^{\cdot-}$, to yield less volatile offspring. Contrary to the gas-phase chemistry, which is well understood due to numerous smog-chamber experiments, aqueous-phase reactions of isoprene are still in their infancy owing to high isoprene hydrophobicity. Sulfate radicals are the major atmospheric actors that underline the phenomenon of an acid rain formation.

Our investigations were carried out in diluted solutions containing sulfate radical anions produced continuously through the bisulfite autoxidation process. The product distribution were monitored *off-line* using UPLC high accuracy (-)electrospray mass spectrometry (Synapt G2 technology, Waters) and HPLC (-)electrospray linear ion trap mass spectrometry (LXQ technology, Thermo Electron). Our experiments revealed the formation of atmospherically important organosulfates, including the MW 212 organosulfate.

IDENTIFICATION OF TANNINS BY HPLC-UV-VIS-ESI MS IN PLANT DYESTUFFS

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Tannins are complex mixtures of polyphenolic compounds (with molecular masses ranging from 500 up to 20,000 Da), which occur in multitude of plants. They may be classified as hydrolyzable tannins (gallic and ellagic acid esters) and non-hydrolyzable (condensed) tannins or proanthocyanidins. Their structures allow them to crosslink other molecules by binding via different phenolic groups and forming large aggregates. Moreover, they exhibit strong affinity to the cellulose fibers, which makes them useful not only for dyeing, but primarily for mordanting plant fibers.

Variety of tannins found in natural dyes as well as their complex structures makes separation step indispensable in their identification. The most suitable for this purpose is high performance liquid chromatography (HPLC), while a tandem mass spectrometer with electrospray ionization (ESI MS/MS) provides structural information on the individual components of the mixture. In the present study, HPLC–UV-Vis–ESI MS/MS has been used to standardless identification of unknown tannins in extract obtained from natural dyes as well as dyed wool fibers. The separated polyphenols observed by spectrophotometric detector at 280 nm have been identified based on MS/MS spectra registered in the positive as well as negative ion modes with various orifice voltages and collision energies. Characteristic signals obtained after fragmentation of the compounds have provided additional information on the lost neutrals, and thus on the type of moieties forming the tannins.

CHARACTERIZATION OF SYNTHETIC DYES WITH HISTORICAL INTEREST BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH DIODE ARRAY DETECTOR AND HIGH RESOLUTION/TANDEM MASS SPECTROMETRY (HPLC-DAD-MS/MS)

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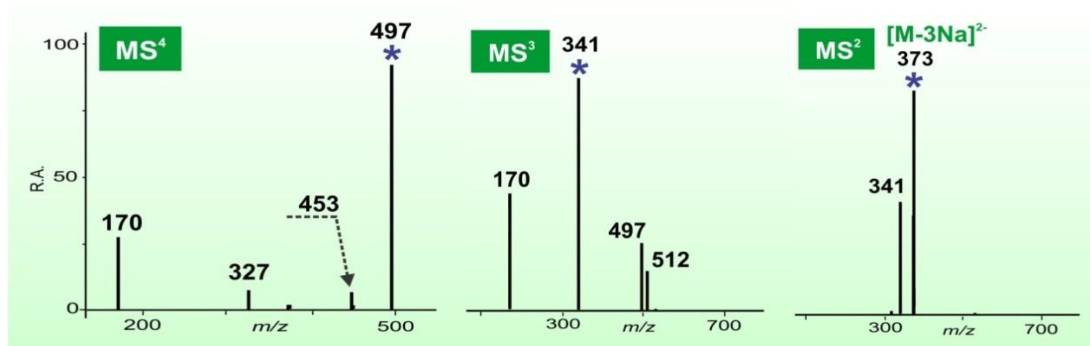
The aims of this study was the characterization of early synthetic colorants used for dyeing threads taken from the manuscript *"Growth of the Dyestuffs Industry: The Application of Science to Art"*, published by R. E. de Rose on the *Journal of Chemical Education*, **1926**, 3: 9, 973-1007.

The dyestuffs were grouped by chemical families and different extraction procedures were performed according to specified groups of dyes. We combined high resolution and multiple-stage tandem mass spectrometry to characterize the chemical composition of different chromophores that constitute the dyes. This technique was coupled with high-performance liquid chromatography, which enabled separation of the analyzed dyes. The negative-ion ESI mode was used for anionic dyes with (poly)sulphonated and/or (poly)sulfated groups whereas non-ionic or cationic dyes were analysed in the positive-ion ESI mode yielding $[M+H]^+$ or molecular adducts in the mass spectra. The detailed interpretation of tandem mass spectra enables the correlation between the effects of the functional groups on fragmentation behaviour. Depending on substituent present in each synthetic dye family, the lost fragments of the examined colorants involved SO_3, SO_2, N_2, $^{\bullet}NO$, NO_2, $^{\bullet}CH_3$, CH_4, NH_3, C_2H_4, C_7H_7, C_7H_9N, $^{\bullet}C_6H_7N$, CO_2, CO, among others, according to previous studies [1].



Tandem Mass Spectra of Light Green SF Yellowish

Acknowledgements

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ACCUMULATION AND ESTIMATION OF HEAVY METALS IN WATER, SEDIMENT AND FISH FROM DANUBE RIVER, ROMANIA

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To estimate the metal concentrations, sediment and fish samples can be considered significant indicators in aquatic ecosystems; therefore, heavy metals exist in low levels in water, but they are found in concerning concentrations in sediments and living organisms. Heavy metals are harmful for plants, aquatic organisms and human health at certain levels of exposure [1], [2].

The purpose of this study was to investigate the heavy metals (manganese, cadminum, nickel, chromium, zinc, iron, lead and copper) and to determine contamination levels of water, sediment [3] and tissues of *Gobius cephalarges* from Cernavoda-Topalu area. The Danube River is important source of agriculture in Romania and also of food and drink for populations, but the quality of this ecosystem has been degrading due to human activities and industry developed in the area. Metals concentrations were measured using graphit furnance atomic absorption spectrophotometer 650 Zeenit, from Analytik Jena. Fish tissues and sediments were extracted using wet digestion method. The quality control of digestion was tested using Certified Reference Material that lead to a recovery of 95-110 %. The method was validated checking a few parameters as: detection limit attained as the minimum concentration detected (Table 1), accuracy using certified reference materials specified for each matrix and recovery [4]. The obtained results for water were compared with national water quality guidelines. The analysis of heavy metals in sediments indicated no exceeded of recommended levels. Fe was maximally accumulated, followed by Ni, Cu, Pb, Mn, Zn, Cd.

Table 1: Detection limit for the elements measured using graphite furnace atomic absorption spectrophotometer and the wavelength used for measurements

Element	Wavelength(nm)	Instrumental detection limit(µg/l)
Cd	228.8	0.013447
Cu	324.8	0.2169
Ni	232	0.484
Pb	283.3	0.23516
Cr	357,9	0.53
Zn	213.9	0.26442

In the *Gobius cephalarges* samples, the heavy metal concentrations were found to decrease as Zn > Fe > Cu > Ni > Mn > Cd > Pb. Heavy metal concentrations in fish samples were found to be above maximum tolerable values provided by national law, so that the fish is safe to be eaten.

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MOLECULAR INSIGHT INTO THE POSTOPERATIVE STATE OF DIABETIC PATIENTS

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The aim of the presented clinical study is to clarify on a molecular level the impact of bariatric surgery treatment on patients who suffer from diabetes mellitus type 2 (T2DM). Along with monitoring of levels of well-known biomarkers of inflammatory processes the other important task has been to identify new potential biomarkers specific for postoperative state of patients with T2DM.

Newly developed methods of analysis taking advantage of combination of high performance liquid chromatography with high-resolution mass spectrometric detection have proven to be an ideal choice for analyses of complex matrices such as urine or blood plasma. The levels of biomarkers of inflammatory processes (aliphatic aldehydes, 8-isoprostane, cysteinyl leukotrienes, o-tyrosine, 3-chlorotyrosine, 3-nitrotyrosine, 8-hydroxyguanosine, 5-(hydroxymethyl)uracil, 8-hydroxy-2'-deoxyguanosin) were significantly elevated speaking of increase as high as 80% in the case of LTB4 and 8-isoprostane. There have been identified structures already associated with diabetes such as L-3-methylhistidine (muscle tissue degradation metabolite) or 2''-Deoxy-N-methyladenosine (insulin excretion signal molecule) as well as novel structures corresponding with the state of the patients, e.g. 2'-deoxy-5'-inosinic acid (hypoxanthine nucleoside, which possibly triggers DNA replication errors), symmetric and asymmetric dimethylarginine (kidneys damage markers), anserine and homocarnosine (endogenic dipeptide antioxidants), acetyl-N-formyl-5-methoxykynurenamine (oxidative stress preventer), 17-octadecynoic acid (inhibitor leukotriene B4 20-hydroxylase and renal CYP450 ω-hydroxylase). Despite the general success of bariatric surgery in the treatment of T2DM the levels of inflammatory processes biomarkers have proven to be significantly elevated even after six months from performed surgery. Some of the novel potential biomarkers exhibit the elevated values as well.

The proper molecular insight into the postoperative changes in metabolic pathways may define a new approach to the treatment of diabetic patients. Deep knowledge of the effects of bariatric surgeries represents the key to the effective and successful treatment making a promise of full life of the patients.

Acknowledgments

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METHOD DEVELOPMENT OF MONITORING OF TRYPTOPHAN AND ITS METABOLITES IN BRAIN TISSUE OF INDIVIDUALS WITH IMMUNE DYSREGULATION AND LATENT TOXOPLASMOSIS

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The aim of presented work was to develop a highly sensitive, accurate and rapid analytical method based on combination of high-efficiency separation by ultra high performance liquid chromatography (UHPLC) and ultra-sensitive and selective detection by high resolution mass spectrometry (HRMS) to determine the concentration levels of tryptophan and its metabolites formed in kynurenine catabolic pathway (kynurenine, kynurenic acid, 3-hydroxykynurenine, quinoline acid, etc.).

Concentration levels of tryptophan catabolic pathway metabolites are strongly influenced by latent toxoplasmosis, immune dysregulation, schizophrenia and other psychopathological disorders. Monitoring of them should help to describe processes in brain during pathological conditions. Besides monitoring of tryptophan and its metabolites there were also monitored concentration levels of selected neurotransmitters - dopamine (DA), serotonin (5-HT), γ aminobutyric acid (GABA) and glutamate (Glu), and their metabolites for clarification of neuropathological processes occurring in the brain due to toxoplasmosis and immune dysregulation.

The developed analytical method consists of separation of analytes from complex biological tissue (blood plasma, brain tissue) by solvent extraction, derivatization of analytes (to significantly decrease the LOD and LOQ values and to stabilize the analytes) and their chromatographic separation combined with mass spectrometric detection.

The individual steps were optimized and the method was validated. Developed method was verified by an animal study comparing the concentration levels of analytes in blood plasma and brain tissue of rat exposed to toxoplasmosis and immune dysregulation to the healthy subjects. All the data obtained from animal studies were statistically evaluated.

There has been observed increase in the concentration levels of tryptophan and its metabolites in the groups with immune dysregulation and latent toxoplasmosis (compared to the control group). Dispersion diagrams component score has been demonstrated in the similarity of individuals in each group and statistically significant difference among them.

Acknowledgments

This work was financially supported by the EU structural funds – “Operational Programme Prague – Competitiveness” (Grant CZ.2.16/3.1.00/22197), by the Ministry of Education, Youth and Sports Czech Republic in program “National Programme of Sustainability I” – NPU I (LO 1215) (Grant No.: MSMT-34807/2013) and, by the Ministry of Health Czech Republic (grants No. NT 13299).

MOLECULAR APPROACH TO FOCAL CEREBRAL ISCHEMIA

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Hectic lifestyle along with controversial diet habits creates conditions for genesis and rapid progression of so-called diseases of affluence that are difficult to diagnose or treat. Focal cerebral ischemia is one of them. Despite the expectations its incidence is even higher than the ones of cancer or diabetes mellitus, therewithal, with long-term and fatal consequences. The tool we have chosen for online monitoring of ischemia progression is the technique of microdialysis that provides valuable matrix serving to its purpose. The infusion of very potent vasoconstrictor endothelin-1 (ET-1) into rat brain induces ischemia; in collected microdialysate there are quantified amino acids, neurotransmitters and other bioactive molecules.

Prior to the analysis itself the microdialysates underwent the derivatization reaction leading to the significant improvement of limits of detection and limits of quantification. There has been also performed the chiral derivatization of amino acids as an effort to understand the role of D-amino acids in neurochemical processes. Analytes were detected via mass spectrometric detector with high resolution ensuring excellent sensitivity, reproducibility and robustness of analyses. The results show significant differences between young and adult animals suggesting that young brain possesses some strange protective mechanisms. The levels of excitatory amino acids as well as allosteric agonists of NMDA (N-methyl-D-aspartate) receptor confirm its great role in ischemic stroke attack. Conclusions from presented work can contribute to early diagnosis of ischemia as well as its therapy and recovery.

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ASSESSMENT OF HEAVY METALS IN COSMETIC PRODUCTS FOR HEALTH IMPACT

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Cosmetic is defined for the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and includes any article intended for use as a component of cosmetic [1] . The European Union (EU) has also developed a list of more than 1000 compounds that are banned for use in cosmetic manufacturing [2]. Cosmetic products are regulated for health and safety. There are concerns regarding the presence of harmful chemicals, including heavy metals, in these products. The skin of eyelid and lip is most susceptible to eczemas, irritant and allergic contact dermatitis. The metal involved in allergic dermatitis are, in order of incidence Ni, Co and Cr, either taken alone or in their association [3]. The quantification of chromium, cadmium, lead, mercury, arsenic, nickel, selenium and antimony was performed in our lab by inductively coupled plasma mass-spectrometry (ICP-MS). The analyses were preceded by microwave–assisted acid digestion of a variety of commercially eye shadows, lipsticks, nails polished and skin creams were purchased and prepared in duplicate. The digestion here used a small amount of hydrofluoric acid HF to ensure better consistency and recovery of all the metal, including that which might be protected from other acid attack by a silica particle [4]. Cosmetics contain a variety of components that can be challenging to digest and ICP-MS is a good choice for determination of low concentrations of analytes, allowing of toxic and potentially toxic compounds in cosmetics. Our application has shown good results and demonstrated the successful analysis of four types of cosmetics for toxic elements.

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ANALYSIS OF PLASMA AND MEAT FATTY ACIDS IN TROUT BY GC/MS

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The aim of this study was to develop and validate a GC-MS method to determine the fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) plasma and meat. Gas chromatography coupled to mass spectrometry (GC-MS) was used as an excellent technique for fatty acids identification and quantitation [1].

The fatty acids were extracted from 0.5 mL of plasma by adding 0.5 mL chloroform:methanol 2:1 (v:v) [2]. The solution was shaken vigorously for 30 s, at room temperature. 1 g of trout meat was crushed with 1 g of quartz sand in a ceramic dish and homogenized with 5 mL distilled water. After centrifugation for 5 min, the supernatant was collected and the fatty acids were extracted by using the same solvent extraction conditions as for plasma. The extracts were converted to corresponding FAMEs (fatty acids methyl esters) by esterification of the carboxylic functions with 200 µL methanol/ HCl 3M for 20 min at 80°C. Undecaenoic acid (C11:1) was used as internal standard. The nutritional quality of fish species can be evaluated from the fatty acid profile and by determining the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) proportions (Fig. 1). The GC-MS method developed here is simple and good validation parameters, precision and accuracy, were obtained. The method was applied to study seasonal variation of trout fatty acids.

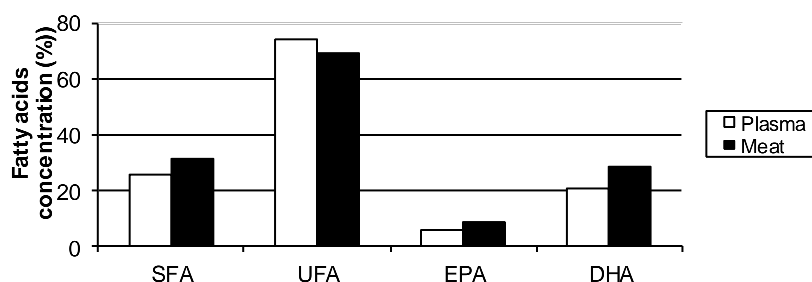

Fig.1 Saturated fatty acids (SFA), unsaturated fatty acids (UFA) and the omega-3 fatty acids: EPA and DHA, identified in trout plasma and meat.

This work was possible due to the financial support of the Sectorial Operational Program for Human Resources Development 2007-2013, co-financed by the European Social Fund, under the project number POSDRU/159/1.5/S/132400 with the title „Young successful researchers – professional development in an international and interdisciplinary environment".

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INTACT SPORE MALDI-TOF MASS SPECTROMETRY OF PUCCINIA PATHOGENIC FUNGI

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Rusts are plant diseases caused by obligate biotrophic fungal pathogens. The most destructive rusts of wheat and other cereals are caused by Puccinia species. Chemical treatments in the crop management reduce yield losses, however this protection is expensive especially for susceptible cultivars. The most economic and ecological way of protection is a breeding and growing of resistant cultivars. Although various resistant cultivars of host crop plants have been cultivated, both the fast development and spread of new virulent races (pathotypes) result in infection of previously resistant crop varieties. Thus there is a need for fast and easy method allowing discovery of newly spreading pathotypes. Recently, intact spore (IS) MALDI-TOF mass spectrometry (MS) was optimized and applied for the identification and differentiation of downy and powdery mildews [1]. It is based on direct measurements of unique peptide/protein profiles from a suspension of intact fungal spores. On the basis of our previous work [1], we started to optimize protocol for IS MALDI-TOF MS of rusts. All optimizations were performed with Puccinia triticina, the most significant rust in our country. Other experiments were done with Puccinia graminis and Puccinia striiformis including their pathotypes. Due to the different morphology of rust spores (size, colour and cell wall surface) it was necessary to look for an optimal solvent composition, select a proper MALDI matrix compound, and evaluate various sample preparation techniques. Spores vary from yellow through orange-red to brown colour. This fact may contribute negatively to the quality of results. In order to improve mass spectra, we evaluated different washing solvent systems with combinations of acetonitrile and organic acids before the application of spores onto MALDI target [2]. Sinapinic acid, ferulic acid, α-cyano-4-hydroxycinnamic acid and caffeic acid were chosen as matrices available for laser desorption and ionization of intact proteins. They were applied individually or in defined combinations. The best protein profiles were finally measured after a washing with acetonitrile: 0.1% TFA (7:3, v/v) and using ferulic acid and sinapic acid as matrices dissolved in a weight ratio of 1:3 in acetonitrile: 2.5% TFA (7:3, v/v). The two-layer volume technique was found the most suitable for the deposition of spores and matrix on the target. Future plans include acquiring peptide/protein profiles from various pathotypes of different Puccinia species in order to find a linkage between their profile patterns and virulence.

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BIOIMAGING OF FERRIFORMS OF SIDEROPHORES IN LUNG TISSUES BY LA-ICP-MS

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Invasive pulmonary aspergillosis (IPA) caused by ubiquitous filamentous fungus Aspergillus fumigatus is a fatal lung disease affecting particularly immuno-compromised patients. Its occurrence is more than 200,000 cases worldwide per year, with an associated mortality rate of 30 - 90 % [1]. Iron, considered as an essential nutrient, plays a key role during the virulence of pathogen microorganisms. In order to overcome the restricted bioavailability of free iron in a host organism, the Aspergillus fumigatus has evolved sophisticated iron acquisition system based on the biosynthesis and excretion of hydroxamate-type siderophores, which are employed in iron delivery from hem in hemoglobin and other host iron-binding proteins (like transferrin, lactoferrin, ferritin). The unique role of hydroxamate siderophores during pathogen virulence defines their potential application as emerging infection disease biomarkers in IPA context.

In this contribution, the objective is the application of LA-ICP-MS to investigate the lateral distribution of iron bound into siderophore complexes across control and infected lung tissue sections coming from Aspergillus infection rat model [2]. Analysis of iron, exactly its 56Fe and 57Fe isotopes, was carried out by the excimer laser ablation system Analyte G2 (Photon Machines, USA) coupled to a quadrupole ICP-MS spectrometer (7700x, Agilent, Japan) equipped with an octopole reaction system working in helium mode to overcome spectral interferences observed mainly on 56Fe. The raw data processing and statistical evaluation of reconstructed iron distribution maps were performed by ImageLab multisensor imaging software and the acquired results were correlated with the corresponding histology.

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CHANGES OF PROTEIN GLYCOSYLATION IN THE COURSE OF RADIOTHERAPY

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The response of organisms to radiation is studied, predominantly, on the genome level. The effect of radiation on proteins and in particular post-translational modifications of proteins is much less understood. Glycosylation is one of the most important and most common post-translational modification of proteins. To our knowledge the relationship between glycosylation and irradiation was studied in one case only; on mice and using relatively large radiation doses [1]. We have performed the first human study, analyzing the effect of radiotherapy on the glycosylation of plasma proteins.

The site-specific glycosylation pattern of blood plasma proteins was analyzed from human samples, using established protocols [2,3]. Samples were collected from patients, suffering from squamous cell carcinoma, before, during and one month after radiotherapy treatment.

Significant changes in glycosylation of various proteins were found. On average, irradiation caused 20-30% changes in glycoform abundances. Not only glycoform abundances, but the effect of radiotherapy also showed large inter-individual variability. In general, glycosylation changes due to radiotherapy last for a long time. Even one month after finishing radiotherapy the induced changes were still present; although most glycoform abundances were started to return to their pre-radiation values.

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LIPID PROFILE CHANGES IN HYPOXIC-ISCHEMIC NEONATAL RAT BRAIN IDENTIFIED BY IMAGING MASS SPECTROMETRY

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Central nervous system tissue has a large abundance of various lipid classes including neutral lipids (cholesterol and acylglycerols), glycolipids (galactosylceramide and gangliosides), and phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingolipids) that are the major building components of intracellular membranes (~25%) [1]. Hypoxic-ischemic (HI) injury of the neonatal brain developed due to impaired cerebral blood flow and decreased oxygen availability evolves with the respect to time and comprise primary energy metabolism failure, reoxygenation and secondary metabolism energy failure. This triggers a cascade of molecular mechanisms including decrease of ATP level, membrane depolarization, cytosolic calcium overload and excitotoxicity, oxidative stress and lipid peroxidation that lead to neuronal cell death (apoptosis, autophagy and necrosis) and inflammation. Increased activities of phospholipases caused by increased intracellular calcium ions concentration and lipid peroxidation induce changes in membrane phospholipid composition and initiate the production of second messengers as arachidonic acid, free fatty acids and free radicals resulting in neuronal death [2, 3]. The aim of the present study is to identify lipid profile changes in hypoxic-ischemic neonatal brain in various times after HI insult by MALDI imaging mass spectrometry which makes possible to identify phospholipids changes between affected and non-affected brain regions directly from tissue, and to localize potential lipid biomarker(s) of HI injury.

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LATERAL RESOLUTION OF DESORPTION NANOELECTROSPRAY

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Desorption nanoelectrospray ionization (nanoDESI) was introduced in 2007 [1,2] as a modification of well-known ambient ionization technique – desorption electrospray ionization (DESI) [3]. None or only minimal sample preparation is carried out for both techniques. NanoDESI characterization demonstrated similarity of internal energy distribution with DESI as well as tolerance to salt in a sample [4]. To improve application potential of nanoDESI, its fourth more robust and user friendly version was introduced. Its use in mass spectrometry imaging has been tested and the lateral resolution evaluated.

Glass targets were coated by rhodamine B and lines were produced on the surface by laser beam. The diameter of laser beam was set at 200 µm and rhodamine B lines (100, 500 and 1000 µm) with 200 µm gaps were created.

A newly constructed device allowing motorized and controlled movement of a sample was used to scan line patterns by nanoDESI. Continuous (5, 25 and 50 µm/s) or discrete (step 25 µm) movement was applied. The slowest movement (5 µm/s) provided the best lateral resolution (bellow 100 µm) but of course the longest analysis time. Similarly to static experiments, nanoDESI imaging is tolerant to high amount of salt in a sample. Although static nanoESI capillaries contained limited volume (15 µl) of spraying liquid, surfaces could be scanned longer than 30 minutes. NanoDESI applicability in MSI was proved.

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DETERMINATION OF PAHS CONTENT IN CONCRETE AND MINERAL OILS USING UPLC/MS METHOD

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A new screening and semi-quantitative approach has been developed for analysis of polycyclic aromatic hydrocarbons (PAHs) in concrete, mortar end mineral oil matrices using atmospheric pressure solid analysis probe (ASAP) triple quadrupole mass spectrometry and ultraperformance liquid chromatography with a photodiode array detector (UPLC- PDA) method. Although phenolic compounds and BETX compounds (benzene, ethylbenzene, toluene, xylene) were found in those samples, PAHs are of particular interest in environmental science because they have well-known carcinogenic and mutagenic effects. The concrete, mortar end mineral oil matrices were prepared through sample preparation from real environmental samples, residential buildings concrete, mortar and mineral oils which were used in construction. The solid phase preparation (SPE) is excluded from sample preparation because we tended to found all different classes of organic contaminants and the relation in pollutants distribution in these constructing materials. The instrumental LODs determined were 0.01–0.04 μg/ml for PAHs. The instrumental parameters were optimized for the analysis of all these compounds, without rigorous sample treatment. Standard for calibration was calibration mix of 16 US EPA PAHs and thirteen of them were detected and quantified, except dibenz[*a*,*h*]anthracene, benzo[*ghi*]perylene and indeno(1,2,3-cd)pyrene.

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GENERAL PROFILE OF PEPTIDES AND LIPIDS IN RAT KIDNEY

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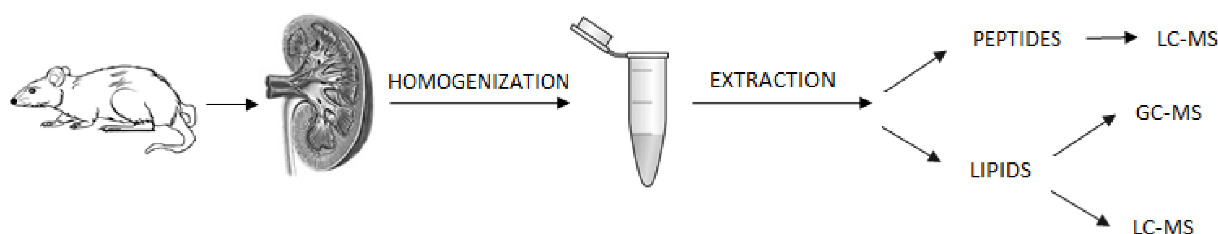
Soft ionization methods, such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), revolutionized the field of mass spectrometry especially by extending its domain into the life sciences [1]. Application of mass spectrometry techniques for biological molecules allowed identification and relative quantitation of specific compounds, such as peptides or lipids in fluids and tissues, to uncover the differences in their structure and concentration levels between the healthy state and a particular disease [2].

The aim of our study was to create a general profile of small molecules in rat kidney, as an useful tool for identification of biomarkers. Peptides and lipids were extracted from homogenized rat kidney tissue. Peptides were separated and analysed on HPLC coupled with Time Of Flight mass analyzer. Lipids were analysed by LC-MS and also by GC-MS after derivatization.



In the future we intend to combine our method of profiling of peptides and lipids with mass spectrometry imaging (MSI) to determine the spacial distribution of these compounds in tissue sections.

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CHARACTERISATION OF HYDROLYSIS PRODUCTS OF AURANOFIN BY ESI -MS

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Auranofin (AUF), namely 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosato- S-(triethyl-phosphine) gold(I), was introduced in the late 1970s as a drug (DMARD) for the treatment of rheumatoid arthritis (RA). Despite the fact that the drug has been used for more than 40 years, its mode of action is still not fully understood and appearing side effects remain unexplained. By understanding the metabolism pathway of the drug the side effects can be avoided and new advances in its medical use can be provided. Auranofin was also tested for treatment of tumors and was found to be more effective than cisplatin in the case of cancer cells of leukemia and lung cancer; also was found to improve the treatment of HIV [1].

Auranofin acts as a prodrug, i.e., its ligands are exchanged by endogenously available thiol ligands, which might affect its reactivity and behavior at the site of action. Typically, Auranofin is administrated orally. Most of the drug is excreted and only 25% is absorbed in the gastrointestinal tract. This drug is firstly located in the acidic environment of the gastrointestinal tract and then into the blood environment with a pH near neutral. It is therefore important to examine the behavior of the gold complex in both acidic and neutral environment in aim to characterize metabolic path of this drug [2]. Electrochemical reaction cell can be simple and fast technique to provide preliminary information about possible tracks for drug transformation.

Experimental conditions were chosen following physiological environment. Samples were prepared in two types of solutions: formic acid pH 4.0 corresponding to gastric acid and ammonium formate, pH 7.4 corresponding to pancreatic juice and to blood. ESI MS/MS was used for the characterization of gold species in water:acetonitrile solutions of Auranofin.

In the positive ion mode, at pH 7.4, five major species were identified in the mass spectra: (a) $[Au\text{-}(PEt_3)_2]^+$ at m/z 433, (b) $[N{\equiv}C\text{-}Au\text{-}PEt_3 + Na]^+$ at m/z 364, (c) $[HS\text{-}Au\text{-} PEt_3 + 2H]^{2+}$ at m/z 175, (d and e) sodiated Auranofin ions at m/z 659 and at m/z 617 obtained *via* loss of acetyl groups from glucose. Two major species were found in the negative ion mode: $[Au\text{-}(PEt_3)_2 - 3H]^{2-}$ at m/z 215 and Auranofin anion at m/z 586 obtained *via* loss of triethylphosphine group (PEt_3).

For acidic media, three main positive ions were obtained at m/z 433, 364, and at m/z 179 obtained *via* loss of acetyl groups and PEt_3. Similar effect was observed in negative ion mode.

Formic acid was found to induce mainly loss of acetyl groups and PEt_3. Neutral media was inducing loss of glucopyranose leading to creation of more active gold-compound.

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PROBING OF AURANOFIN METABOLISM BY SINGLE COMPOUND EC-MS

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Last decade, gold compounds occupy a relevant position constituting a promising class of experimental anticancer metallodrugs. Several research efforts have been devoted to the investigations of the pharmacological properties of gold(I) complexes bearing phosphine ligands, such as the antiarthritic drug - auranofin (2,3,4,6-tetra-O-acetyl-1-thio-b-Dglucopyranosato-S-(triethyl-phosphine) gold(I)), that has also been shown to produce anticancer effects in vitro [1]. To explore the mechanism of the drug metabolism, its behavior in simulated physiological conditions should be examined. Studies carried out in vivo or even in vitro can be economically and time consuming. In aim to reduce costs of drug characterization the preliminary studies can be carried out in the simulated physiological environment with special respect to pH. The oxidation or reduction can be induced by addition of specific reactive species which may interfere ESI MS signals. Another method – without interfering chemical composition - is application of electrochemical reaction chamber Antec's Roxy Reactor Cell (EC), which allows to obtain redox reaction at varied potentials.

The ROXY cell generates metabolites of drugs or xenobiotics, similar to those generated during in vivo metabolic processes, in a significantly shorter time span. The Reactor Cell is a universal flow cell, ideally suited for oxidation, reduction and/or activation of compounds that pass through the cell. The ROXY Potentiostat is based on state-of-the-art electronics with a large voltage range of ± 4.9 V. The generation of specific oxidation products, e.g., metabolites, cleavage products, etc., and supreme control of any conceivable Redox reaction is assured.

Structure of the drugs at the varied redox potential applied in EC have been studied by mass spectrometry with electrospray ionization. The metallodrug analysis was performed in a buffered solution of ammonium formate at pH 7.4, the physiological pH of blood and pancreatic juice. The analysis was conducted three times with gradually increasing oxidation potential. In the positive ion mode of ESI MS, many species were observed already due to hydrolysis of Auranofin. However, few new signals were observed due to more advanced decomposition of acetylated glucose $[Au\text{-}(PEt_3)^2]^+$ at m/z 433, its nitrile adduct at m/z 364 and nitrile adduct with one triethyl-phosphine. At low potentials signals corresponding to hydrolysis were mainly observed at m/z 581 m/z 356 and at m/z 256.

In the negative ion mode signal at m/z 215 was already observed at low potentials and it grows on during analysis - $[Au\text{-}(PEt_3)^2 - 3H]^{2-}$.

It can be concluded that loss of both ligands: glucose and triethyl-phosphine can be expected during drug metabolism and drug activity strictly depends on gold ion activity.

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THE INFLUENCE OF ELECTROCHEMICAL REACTION CHAMBER ON STABILITY OF GOLD(III) COMPLEX

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Gold compounds constitute an emerging class of biologically active substances of special interest as potential anticancer agents. During the past decade a number of structurally diverse gold complexes were reported to be acceptably stable under physiological like conditions and to manifest very promising cytotoxic effects against selected human tumour cell lines, making them good candidates as anti-tumour drugs. There is considerable interest in understanding the precise biochemical mechanisms of these novel cytotoxic agents. Based on experimental evidence collected so far it was hypothesized that these metallodrugs, at variance with classical platinum(II) drugs, produce in most cases growth inhibition effects for tumour through a variety of "DNA-independent" mechanisms.

Aubipy^c [(bipy^{dmb}-H)Au(OH)][PF_6] (where bipy^{dmb}-H = deprotonated 6-(1,1dimethylbenzyl)-2,2′-bipyridine) is a promising gold(III) compound that was characterized chemically and biologically through a few recent studies [21,22]. It consists of a square planar gold(III) center receiving three donors – i.e. C, N, N – from the tridentate bipyridyl ligand while the fourth coordination position is occupied by a hydroxide group (Fig. 1); the latter is the preferential site for ligand replacement reaction and for protein binding. Aubipy^c was found acceptably stable under physiological conditions even in the presence of reducing agents [1].

The electrochemical reaction chamber Antec's Roxy Reactor Cell (EC) was used to generate metabolites of gold(III) complex. The Reactor Cell is a universal flow cell, ideally suited for oxidation, reduction and/or activation of compounds that pass through the cell. The ROXY Potentiostat is based on state-of-the-art electronics with a large voltage range of ± 4.9 V. The generation of specific oxidation products, e.g., metabolites, cleavage products, etc., and supreme control of any conceivable Redox reaction is assured.

Structures of the drug's metabolites have been studied by the mass spectrometry with electrospray ionization. Drugs analysis was performed in a buffered solution of ammonium formate at pH 7.4, the physiological pH of blood and pancreatic juice. Aubipyc was found to be stable compound, which creates only few minor metabolites. Mass spectrum in positive ion mode consisted of only one main signal: [Aubipy^c + OH + Na]$^+$ at m/z 527. No signal was observed in negative ion mode. When varied redox potentials were appliedin ROXY cell, new main signal in ESI mass spectra was obtained at m/z 273 corresponding to [Au – C_6H_4]$^+$. The electrochemical chamber allows for fast and simple analysis of potential drug's metabolites at preliminary stage of drug's studies.

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MASS SPECTROMETRY AS A RESEARCH TOOL FOR ANALYSIS OF MODIFIED PEPTIDES CONTAINING OXIDIZED THREONINE

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The Reactive Oxygen Species (ROS) production is the consequence of an aerobic metabolism. The impaired balance between production and removing of ROS is an undesirable phenomenon that can lead to protein oxidation often resulting in formation of carbonylated derivatives with reactive aldehyde or ketone groups. This post-translational modification is linked to numerous diseases including neurodegenerative, diabetes and cancer. An example of this modification is the oxidized threonine-Thr(O) residue. Hence the great interest in this problem and the resulting need for studies on carbonylated species.[1]

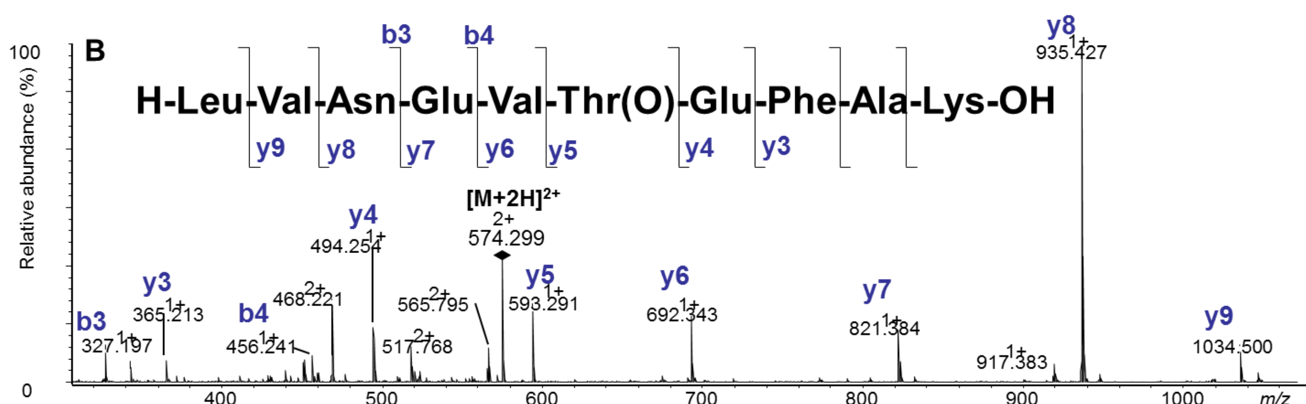

Figure 1. Fragmentation spectrum (ESI-MS/MS) of carbonylated peptide containing oxidized threonine (the sequence shown over the spectrum).

We developed a synthesis of a novel unnatural amino acid Fmoc-Atda-OH [Fmoc Amino(2,5,5-trimetyhyl-1,3-dioxolan-2-yl)acetic acid]- the protected form of oxidized threonine and then applied in solid-phase peptide synthesis of carbonylated peptides using standard Fmoc protocol. Mass spectrometry is a highly versatile and widely used method for the characterization of PTMs. Therefore, the obtained structures were confirmed using ESI-MS as well as it were carried out fragmentation experiments. The use of stable isotope labeling combined with high-resolution mass spectrometry makes an efficient research tool for analysis of glycated and carbonylated peptides or proteins.[2,3] Thus, we have applied ^{18}O labeling approach to qualitative analysis of peptides containing carbonyl moiety.

Acknowledgments:

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APPLICATION OF SPME-LC/MS^n FOR METABOLOMICS DRUG MONITORING IN BIOLOGICAL SAMPLES

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Metabolomics applications require sample preparation methods that are fast, reproducible and able to extract a wide range of analytes of differing polarities. The measurement of drug levels in biological fluids is the corner stone for drug discovery and development as well as for pharmacodynamic, pharmacokinetic studies and drug monitoring. The most relevant matrices to be analyzed for this purpose are plasma or blood, due to providing a good correlation between their concentration and pharmacological effects. Sample preparation is frequently done *"off–line"* and this is often a limiting step to perform fast bioanalysis. The introduction of *"on–line"* sample pretreatment would greatly speed up the analyses. The choice of an appropriate sample–extraction technique is very important step for metabolomic studies. For metabolomic analyses, the analytical technique must therefore be really suitable for a diverse range of small endogenous metabolites in various concentrations. *In vivo* solid phase microextraction (SPME) provides an interesting new complement to the range of technologies currently being employed for *in vivo* analysis of living systems. SPME can match the selectivity and sensitivity for improved time resolution of sampling.

In this study, polypyrrole (PPy) and polythiophene (PTh) SPME coatings were used and evaluated their ability to extract selected drugs from different classes with different physico–chemical properties and of widely varying polarities. The SPME coatings were evaluated by analyzing clinically relevant antibiotic drugs – linezolid, daptomycin, amoxicillin and moxifloxacin. Important factors in the optimization of SPME efficiency such as extraction time, temperature, pH of the matrix, influence of anticoagulants on sorption mechanisms, and desorption conditions are discussed. The PPy film displayed high extraction efficiency (selectivity and sensibility) towards the target analytes among studied SPME coatings. The results demonstrate the potential of *in vivo* SPME as a useful sample preparation tool for chromatographic based metabolomics drug monitoring in the biomedical application from patients receiving therapeutic dosages.

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A NEW METHOD OF OXYGEN-18 LABELING OF AMINO ACIDS OR PEPTIDES IN QUANTINATIVE PROTEOMICS

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The use of mass spectrometry techniques in quantitative proteomics has many advantages. However we need to apply some standards for absolute quantification of proteins, as different molecules often have different ionization efficiencies. To that end, one of the methods is using isotopically labeled synthetic peptides. A simple and relatively low cost approach is to use oxygen-18 isotope. Currently the most widespread method is enzymatic ^{18}O-labeling. It relies on postdigestion reincubation of the peptide with protease in buffers that contain water with oxygen-18. The exchange is possible on the C-terminal carboxyl group which fits the specificity of the enzyme. Despite development of this approach there are still disadvantages like back-exchange (present even after quenching the enzymatic hydrolysis reaction) and a limited selectivity of labeling.[1,2]

We have designed a new method which can be used to label peptides using a small amount of $H_2{}^{18}O$ with a high efficiency of exchange of oxygen atoms, thus reducing the cost of labeling. In our strategy we employed a mixture of dry HCl-saturated 1,4-dioxane and water labeled with oxygen-18 (97% ^{18}O), and the reaction was conducted in a microwave synthesizer. This method can be utilized for Fmoc-protected amino acids, which can then be stereospecifically introduced to the peptide at the synthesis step. The same approach can be also applied for labeling peptides assembled on Wang resin. In this case the HCl-saturated dioxane containing $H_2{}^{18}O$ cleaves the peptide from the resin, deprotects the amino acids' side chains and at the same time incorporates ^{18}O atoms to the carboxylic groups in the peptide molecule.[3]

Acknowledgments:
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THE INFLUENCE OF PLATINUM(II) ION ON FRAGMENTATION MECHANISM OF PEPTIDES COMPLEXED WITH PLATINUM(II)

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One of the methods for obtaining structural information of the compounds by mass spectrometry technique, is the collision-induced dissociation (CID) usually coupled with electrospray ionization (ESI). ESI-MS/MS has been widely used in inorganic [1], organometallic [2] and bioanalytical [3] chemistry, especially in determination of peptides and proteins sequence. Fragmentation of peptides usually occurs in the main polypeptide chain and a series of distinctive fragment ions are generated, on the basis of which the amino acids sequence can be identified. Unfortunately, cleavage of side-chains can also occur, which results in additional series of ions that often hinder determination of the amino acid sequence of the investigated peptide. However, number of rules have been established, which facilitate the interpretation of the MS/MS spectra of peptides, enabling the proper identification of the peptide sequence. In our study, we would like to check if these rules can also be applied for the interpretation of the MS/MS spectra of peptides complexed with platinum(II), due to the specific properties of platinum(II) ion. Moreover, by comparing the fragmentation spectra of the complexed and uncomplexed peptides, we will try to determine the exact binding site of the platinum(II) ion to the peptide. Obtained data can be useful for medicine and biochemistry studies, because platinum is known to participate in inhibition of tumor growth and the peptide-platin drugs can be used in cancer treatment [4].

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RADICAL ANIONS FORMATION IN THE GAS PHASE ADDITION-ELIMINATION REACTION OF PHENIDE IONS WITH ALDEHYDES

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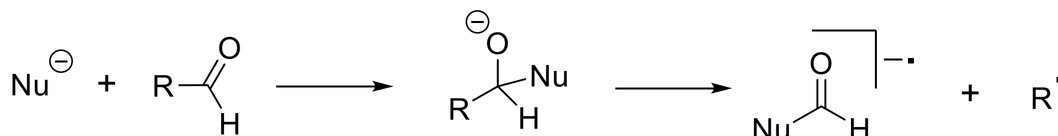
Mass Spectrometry Team in the Institute of Organic Chemistry PAS have developed simple and efficient method for generating gas-phase phenide ions with various substituents in the ring.[1] We are studying the reactions of these anions with various gas-phase reagents, which are introduced with the curtain or collision gases. In this presentation, the results of the gas-phase reactions of substituted phenide ions with aldehydes are reported.



Scheme 1

The addition-elimination reaction to the carbonyl group is very common and useful reaction in the organic chemistry. A typical addition-elimination reaction runs through two steps: at first, an attack of a nucleophile to the carbonyl group occurs and then, in the second step, an elimination of the anionic leaving group takes place. In our case we observed homolytic cleavage and elimination of radicals in the second step of the reaction (Scheme 1).

The aim of this work was to determine thermochemistry of reactions of carbanions with aldehydes in the gas phase and to compare obtained experimental results with thermochemical calculations. DFT calculations were used to create energy profiles of reactions of carbanions with pivalic aldehyde and furfural. It has been found that the formation of radical anion is advantageous in terms of thermochemistry only for the reactions of carbanions with pivalic aldehyde. Energy profiles of the particular reactions correlate well with experimental results.

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ANALYSIS OF NICOTIANAMINE COMPLEXES BY MEANS OF HILIC ESI MS/MS

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The soil contamination with metals is considered as a one of the most important environmental problems reflecting human health. Phytoremediation – usage of plants in soil cleaning – can be applied when mechanism of metal uptake, translocation and accumulation will be well understood [1]. Nicotianamine (NA)- a non-proteinogenic amino acid is one of the ubiquitous metal chelators in plants [2]. It was found to be an important bioligand in long-distance metal transport [3]. In order to get further information about participation of NA in metal chelation and transport from root to the shoot unambiguous identification methods are necessary.

Separated roots and shoots from hydroponically grown tomatoes were frozen, lyophilised and ground. Extraction was carried out for 30 min with Tris-HCl pH 7.5 (supported by shaking or sonication). Subsequently the sample was centrifuged (4°C, 10000 rpm, 15min) and the supernatant was filtered with syringe filter 0.2µm. The *in vitro* complexes were prepared in Tris-HCl pH 7.4 with standard solution of nicotianamine (10 ppm) and metals with molar ration 1:1. The mobile phase in gradient HILIC separation were mixtures: water-acetonitrile (95%) with 5 mM ammonium acetate pH 7.5 and water solution of ammonium acetate pH 7.5. In order to get the certain information about the identity of the complex by ESI MS/MS the preliminary studies were performed using mixtures of standards and to obtain product ion mass spectra and to select optimal conditions for appropriate pairs of parent and product ions.

Analysis of *in vitro* nicotianamine-metal complexes was performed by means of high resolution MS [2] and tandem mass spectrometry (ESI MS/MS) in Ni-hyperaccumulator *Thlaspi arvensae* [4]. Our studies indicate that high resolution mass spectrometry is not the only way to get information about NA-complexes obtained *in vitro* and present in plant extracts. To the best of our knowledge this is the first time of use the MRM mode of ion scanning in order to identify NA complexes with various metal in plant extract. Multiple reaction monitoring mode allowed to distinguish NA-complexes with Zn, Cu and Cd in spite of their small concentration in plant tissues – below 10µg g^{-1}. The drawback of presented method is the necessity of usage of standard substances to establish appropriate instrument parameters for both identification an quantification.

Hydrophilic interaction liquid chromatography was found to be most appropriate to separate nicotianamine complexes in plant extracts from plant tissue. The detection of nicotianamine complexes with various metals both *in vitro* and in tomato extracts can be identified by means of ESI MS/MS with multiple reaction monitoring.

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ION MOBILITY MASS SPECTROMETRY OF DIMETHYL EPHEDRINIUM BROMIDE (DMEB) SUPRAMOLECULAR AGGREGATES

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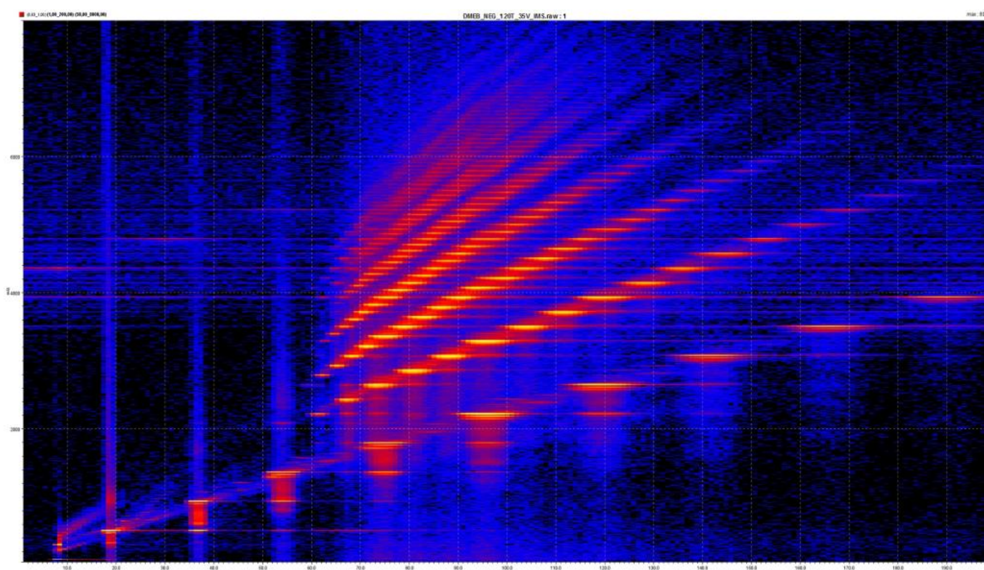
Dimethyl ammonium bromide (DMEB) is a cationic surfactant used as chiral phase catalyst and micellar microenvironment for enantioselective reactions. The formation of giant vesicles of DMED has also been evidenced, in condensed phase [1]. These can be used as model of biologic membranes. Being we strongly interested into the potential capacity to form large aggregates and to eventually incorporate enantioselectively chiral substances in gas phase, we started our investigation on this molecule.

This preliminary information deals with the self-assembling properties of DMEB during ESI ionization, and, in order to gain a deeper knowledge, the aggregates formed were analyzed by mass spectrometry and Ion Mobility Mass Spectrometry (IMMS).

It has been previously observed for some anionic and cationic surfactants that the aggregation process is specific and leads to the formation of aggregates with a well defined shape [2], the same also applies for DMEB. It is also interesting to note that a proportional and regular increase in charge is observed on increasing the size of DMEB aggregates. This is a further evidence of ordered objects characterized by a regular and well defined structure.

Aggregates of DMEB especially in negative ion mode confirm their tendency to form large systems. Indeed aggregates containing up to 130 monomers and 8 charges were easily observed.



HDMS view in DriftScope of singly and multiply charged DMEB aggregates in negative ion mode

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GC/MS PROFILING OF FFPE TISSUES FOR SCREENING POTENTIAL METABOLOMIC BIOMARKERS OF DIFFERENT THYROID CANCER TYPES

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Thyroid cancer is the most common endocrine malignancy. On the basis of histopathological features there are distinguished four main types of thyroid carcinoma: papillary, follicular, medullary and anaplastic. Determining the type of thyroid cancer is crucial for the assessment of prognosis and treatment selection. Unfortunately in some cases, appropriate classification of cancer is not possible only based on histopathological features. Therefore such classification can be supported by the molecular biomarkers identified with the use of different „omics" techniques. Next to genomics, transcriptomics and proteomics, metabolomic approach can be applied to reveal differences between various types of thyroid cancer and to identify new potential molecular biomarkers. Recent works have shown significant metabolomic differences present in normal and thyroid tissues. However, there still have been no attempts made towards delivering novel biomarkers to be implemented in clinical diagnosis and classification of thyroid cancer.

In the present study, gas chromatography/mass spectrometry (GC/MS) metabolic profiling have been applied for searching potential biomarkers of the main thyroid cancer types present in formalin fixed paraffin embedded (FFPE) specimens. An important issue in the diagnostics of thyroid cancer is differentiation between follicular adenoma, follicular carcinoma and the follicular variant of papillary carcinoma. Multivariate statistical analysis provided discrimination between those types of thyroid lesions. The differences in the metabolomic profiles allowed the identification of the most important metabolites for different thyroid cancer types. Metabolic changes in various thyroid cancer types were mainly related with lipids (fatty acids and their esters), carboxylic acids (lactic, citric, oxalic, succinic acid) and gluconic acid. Mio-inositol phosphate were found to be specific biomarker to distinguish follicular adenoma from thyroid carcinoma. These results demonstrate the utilization of the GC/MS-based analysis of extracts from formalin fixed paraffin embedded thyroid tissue as the potentially auxiliary diagnostic tool of thyroid lesions.

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WHOLE BODY RESPONSE TO RADIATION IN HEAD AND NECK AND PROSTATE CANCER PATIENT; THE SERUM PROTEOME COMPARATIVE ANALYSIS

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Here we compared effects associated with local body irradiation during RT of two solid tumors: head & neck cancer (HNC) and prostate cancer (PC). About 120 HNC and 120 PC patients were enrolled into the study. All patients were subjected to radical IMRT with maximum GTV doses in the range 50-73.8 Gy and 74-76 Gy, respectively. Acute mucosal toxicity and gastrointestinal/genitourinary toxicity was assessed. Three consecutive blood samples were collected before, during and after RT. The endogenous serum peptidome was profiled using MALDI mass spectrometry. Furthermore, serum proteins were analyzed using label-free LC-MS/MS "shotgun" approach in complete sets of samples representing 20 patients from each cancer group.

In case of HNC patients radiation-induced changes in serum peptidome accumulated constantly during the treatment and their highest level was detected soon after the end of RT (~60% of components changed their abundance at significance level $p<0.0001$ between pre- and post-exposure samples). Moreover, changes in serum peptidome correlated significantly with intensity of acute mucosal reactions and volume of normal tissue irradiated with low-to-medium doses. In contrast, in case of PC patients majority of radiation-induced changes were detected 2-3 weeks after start of RT and their extend was less significant (~30% of components changed their abundance at significance level $p<0.05$). Furthermore, correlations between changes in serum peptidome and escalation of radiation toxicity or volume of tissues irradiated at low/medium doses were not detected. About 200 serum proteins were identified and quantified: ~10% and ~2% of identified proteins changed their abundance between pre- and post-exposure samples from HNC and PC patients, respectively.

The effects of local irradiation were documented at the level of serum proteome, which is an apparent indicator of the patient's whole body response. Significant differences were noted between patients irradiated because of HNC and PC in spite of the fact that similar volumes of tissues were irradiated with similar doses in both groups. This observation presumably reflected differences in radiosensitivity and corresponding early radiation toxicity in normal tissues/organs adjacent to cancer target and exposed to low-to-medium doses during IMRT.

ION MOBILITY AS A PROMISING TOOL TO DEFINITIVELY ACCESS THE MOLECULAR STRUCTURE OF SAPONINS

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For many decades, scientists' effort has been focused on structural characterization of natural molecules. Among all the natural molecules under interest, saponins raise today a great interest due to their bio diversity and their involvement in biological phenomena.

Saponin molecules are secondary metabolites glycoside widespread in plants and marine animals such as Echinoderms. By definition, this family of molecules is constituted of a glycone part (oligosaccharide) covalently attached to the aglycone based on a steroidal or triterpenoidic skeleton. Due to the huge variety in the nature of monosaccharides and steroids, the molecular diversity of saponins is more than important. This diversity is even greater than the oligosaccharide chain may have isomeric structures classified into 3 groups. The first and the second kind of isomers include the saponins with linear or ramified oligosaccharides named mono- and bidesmosidic saponins respectively. The third group includes saponins with the cyclic oligomeric chain attached to the aglycone, affording the so called macrocyclic saponins.

Today, modern techniques of mass spectrometry are most often used to decipher the structure of saponins in very complex mixtures of congeners. For many years, the laboratories involved in the present project actively work to resolve at the molecular level the structure / activity relationship of saponins in sea cucumbers and starfishes. Conventional techniques of mass spectrometry are used to acquire a significant number of data but a large amount of molecules often escape identification essentially because of subtle structural differences between saponins. Recently, ion mobility has been introduced and provides a new approach to the structural characterization of molecules. In the context of this project, we are developing a novel method based on ion mobility to characterize mixtures of saponins from different marine animals. The contribution of theoretical chemistry for the development of our work will be essential and will be achieved in collaboration with theoretical chemists at UMONS.

DIFFERENCES IN SOIL EXTRACTION EFFECTIVENESS FOR DDT AND ITS DEGRADANTS

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The DDT is former one of the most commonly used pesticide, very degradant and is still present in the environment due to the very long half-life time. It can last in soil up to tens of years and its primary degradants DDE, DDD and DDMU are even more stable and have very similar toxic properties.

In this work we investigate the differences in effectiveness of extraction for DDT and the more polar degradants using various solvent mixtures. The highly contaminated soil with DDT samples, with high levels of degradants were collected near the pesticides burial sites where pesticides were stored for many years.

The structural changes like dechlorination and dehydrochlorination strongly affect the properties of the compounds and significantly modify the effectiveness of the extraction process with particular solvent mixtures.

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PESTICIDES DEGRADATION: STORAGE IN SPECIFIED CONDITION IN OPPOSITE TO PRESENCE IN SOIL

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Pesticides have been widely used in agriculture since 1940's. Development in chemical industry caused that many compounds previously used as pesticides were replaced by less hazardous, improved, cheaper or more easily utilized products. Out of use and outdated products were stored in pesticides burial sites which concrete housings, in the course of time, became leaky. In many cases toxic substances from these containers penetrate into the soil and underground waters causing serious contamination of environment.

In this study we compare the composition of the outdated pesticides products with the compounds that were found in samples of soil collected near the pesticides burial sites. We have tried to find the differences that can indicate other degradation paths in these variant conditions, especially the possible impact of bacterial metabolism.

Very stable compounds like DDT and HCH and some less stable compounds like atrazine were investigated. The higher level of degradants were found in soil samples than in products kept in the storage places. The differences are probably the result of the conditions of storage and those in soil, especially in terms of humidity level.

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PROBING DISSOCIATION OF COLLISIONALLY EXCITED NON-COVALENT COMPLEXES BY ENERGY-RESOLVED CID AND COMPUTATIONAL CHEMISTRY

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A huge interest in the cucurbit[n]uril (CB[n], n=5–10) family of molecular containers has surged in recent years because of their outstanding binding capabilities.[1] Cucurbiturils are macrocyclic molecules built from glycoluril repeat units. These pumpkin-shaped molecules have a hydrophobic inner cavity and two identical carbonyl-laced portals and form stable inclusion complexes with various protonated alkyl and aryl amines [1]. Recently, Isaacs et al. reported the kinetically controlled synthesis as well as the recognition properties of a new member of the cucurbituril family, namely nor-seco-cucurbit[10]uril (ns-CB[10], Scheme 1), which results from the formal extrusion of two CH2 bridges from CB[10] along with bond reorganization.[2] This molecular container presents two cavities and is suitable for binding guest molecules to form ternary complexes (2:1). Several key features of the binding capabilities of this large host molecule were then described. In particular, ns-CB[10] was shown to conserve much of the binding properties of the CB[n] molecules. However, this ditopic receptor also: 1) binds larger guests than expected given that both cavities are each shaped by only five glycoluril residues, thus highlighting the structural responsiveness of the cavity; and 2) displays homotropic allostery based on a guest-size-induced preorganization mechanism.

Nowadays, MS is more and more used to study noncovalent interactions exctracted from the solutions to the gas phase by means of the electrospray ionization source (ESI-source). In addition to numerous applications in analytical sciences, mass spectrometry is known as a powerful tool for the determination of intrinsic thermodynamic properties of gaseous ions. In that area, collision-induced dissociation (CID) experiments afford accurate activation energies values for kinetically controlled reactions [3] by the survival yied (SY) method. This method links the amount of a precursor ion through his fragments to the internal energy (E_{int}) of the precursor ion.

A previous article written by our research group reported in details a joint experimental and theoretical study by mass spectrometry (MS) and computational chemistry of the binding properties of ns-CB[10] towards selected protonated alkyl and aryl amines. The underlying idea lied in a much broader context, which aimed at exploring the potentialities of MS for the study of supramolecular chemistry [4]. Back then, MS analysis allowed us to identify doubly and triply charged ternary complexes (2 :1) for each studied guest.

The purpose is now to study the gas phase decomplexation processes for those ternary comlplexes by both kinetics and thermodynamics approaches. The decomplexation order for complexes between ns-CB[10] and diamnophenylene, para-toluidine, para-xylylenediamine and adamantylamine is not the same depending of the charge state of the studied complexes. In the case of doubly charged compounds, adamantylamine formed the weakest complex while it formed the strongest one for the triply charged coumponds case. The association of both Mass Spectrometry methods and theoretical calculations allowed us to explain a such difference of behavior for ns-CB[10] – adamantylamin complexes. The complete gas phase decomplexation order for the complexes between the bitopic cage and the 4 stutied guests has also been solved by the association of those 2 powerful technics.

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NOTES

Waters
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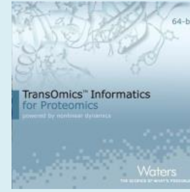

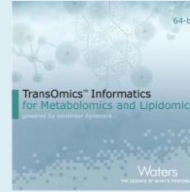

Systemy chromatografii cieczowwej (LC) , spektrometrii mas (MS) oraz laboratoryjne produkty informatyczne i usługi firmy Waters są symbolem jakości i wydajności w laboratoriach analitycznych na całym świecie.
Oferujemy rozwiązania dla branży farmaceutycznej, nauk przyrodniczych i na rynku przemysłowym i ochrony środowiska. Firma Waters z siedzibą w Milford w stanie Massachusetts (USA) jest aktywna na rynkach w 125 krajach , zatrudnia około 6000 osób na całym świecie, a jej produkty wytwarzane są w zakładach zlokalizowanych w Milford w stanie Massachusetts (USA) , Wexford w Irlandii oraz Manchesterze w Anglii.

Waters oferuje kompletne rozwiązania analityczne , informatyczne , obsługę serwisową oraz wsparcie merytoryczne i szkoleniowe dla następujących produktów :

1. CHROMATOGRAFY CIECZOWE :

ACQUITY UPLC I/H/M-Class ; ACQUITY UPC^2 ; ACQUITY APC ACQUITY UPLC Systems z technologią 2D ; ACQUITY UPLC M-Class z technologią HDX ; Alliance HPLC

2. SPEKTROMETRY MAS:

SQ Detector2 , ACQUITY QDa (analizatory typu Single Quad) Xevo TQ-S , Xevo TQ-S micro , Xevo TQD (analizatory typu Tandem Quads) ; Xevo G2-XS (Q)TOF (analizatory typu TOF i QTOF); Synapt G2-Si (HD)MS (analizatory typu Quad-TriWave (Ion Mobility Separation) -TOF)

3. SIECI KOMPUTEROWE

4. INFORMATYKA I OPROGRAMOWANIE :

NuGenesis SDMS , NuGenesis ELN;
Empower (Chromatography Data Software and Application Manager) ; Paradigm Scientific Search Software;
UNIFI Scientific Information System; TransOmics Solution with Progenesis Software; Fusion Method Development Software from S-Matrix

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ANCHEM

Aparatura analityczna. Wyposażenie laboratoriów.

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Perlan Technologies